PREVALENCE OF HELICOBACTER PYLORI INFECTION AND INFLUENCE OF LARYNGOPHARYNGEAL REFLUX IN PATIENTS WITH LARYNGEAL PATHOLOGIES

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF M.S BRANCH IV

OTORHINOLARYNGOLOGY EXAMINATION OF THE TAMIL NADU

DR. M.G.R.MEDICAL UNIVERSITY TO BE HELD IN APRIL 2015.

PREVALENCE OF HELICOBACTER PYLORI INFECTION AND INFLUENCE OF LARYNGOPHARYNGEAL REFLUX IN PATIENTS WITH LARYNGEAL PATHOLOGIES

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF M.S BRANCH IV

OTORHINOLARYNGOLOGY EXAMINATION OF THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY TO BE HELD IN APRIL 2015

DEPARTMENT OF OTORHINOLARYNGOLOGY
CHRISTIAN MEDICAL COLLEGE
VELLORE

CERTIFICATE

I declare that this dissertation entitled "**Prevalence of *Helicobacter pylori* infection and influence of laryngopharyngeal reflux in patients with laryngeal pathologies**'' submitted towards fulfilment of the requirements of the Tamil Nadu Dr. M.G.R. Medical University for the MS Branch IV, Otorhinolaryngology examination to be conducted in April 2015, is the bonafide work of Dr. Neenu Anna Joseph, postgraduate student in the Department of Otorhinolaryngology, Christian Medical College, Vellore.

Dr. Neenu Anna Joseph
Postgraduate Student (M S Otorhinolaryngology)
Register Number: 221314354
Department of Otorhinolaryngology
Christian Medical College
Vellore.

DEPARTMENT OF OTORHINOLARYNGOLOGY
CHRISTIAN MEDICAL COLLEGE
VELLORE

CERTIFICATE

This is to certify that the dissertation entitled "**Prevalence of *Helicobacter pylori* infection and influence of laryngopharyngeal reflux in patients with laryngeal pathologies**'' is a bonafide original work of **Dr. Neenu Anna Joseph**, submitted in partial fulfilment of the rules and regulations for the M S Branch IV, Otorhinolaryngology examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in April 2015.

Principal
Christian Medical College
Vellore- 632002
India.

Dr. John Mathew
Professor and Head,
Department of Otorhinolaryngology,
Christian Medical College,
Vellore.

DEPARTMENT OF OTORHINOLARYNGOLOGY
CHRISTIAN MEDICAL COLLEGE
VELLORE

CERTIFICATE

This is to certify that the dissertation entitled "**Prevalence of *Helicobacter pylori* infection and influence of laryngopharyngeal reflux in patients with laryngeal pathologies**'' is a bonafide original work of **Dr. Neenu Anna Joseph**, submitted in partial fulfilment of the rules and regulations for the M S Branch IV, Otorhinolaryngology examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in April 2015.

Dr. Suma Susan Mathews
Professor and Guide,
Department of Otorhinolaryngology,
Christian Medical College,
Vellore.

ANTI-PLAGIARISM CERTIFICATE

turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Neenu Anna Joseph
Assignment title:	TNMGRMU EXAMINATIONS
Submission title:	Prevalence of Helicobacter pylori infec..
File name:	NEENU_ANNA_JOSEPH_THESIS.d...
File size:	7M
Page count:	106
Word count:	17,907
Character count:	99,169
Submission date:	24-Sep-2014 07:40PM
Submission ID:	454477288

INTRODUCTION

ANTI-PLAGIARISM CERTIFICATE

Prevalence of Helicobacter pylori infection in patients with laryngeal pathologies

ORIGINALITY REPORT

22%	12%	18%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.rtog.org Internet Source	1%
2	www.unm.edu Internet Source	1%
3	Beasley, Nigel. "Anatomy of the larynx and tracheobronchial tree", Scott-Brown s Otorhinolaryngology Head and Neck Surgery 7Ed 3 volume set, 2008. Publication	1%
4	McGlashan, Julian. "Disorders of the voice", Scott-Brown s Otorhinolaryngology Head and Neck Surgery 7Ed 3 volume set, 2008. Publication	1%
5	www.clinicalpublishing.co.uk Internet Source	1%
6	Peter C. Belafsky. "The Validity and Reliability of the Reflux Finding Score (RFS)", The Laryngoscope, 08/2001 Publication	1%
7	www.acpmedicine.com Internet Source	1%

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Glas) (EDIN)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

October 17, 2013

Dr. Neenu Anna Joseph
PG Registrar
Department of ENT
Christian Medical College
Vellore 632 002

Sub: **Fluid Research grant project:**
Prevalence of *Helicobacter pylori* infection in patients with laryngeal pathologies.
Dr. Neenu Anna Joseph, ENT, Dr. Suma Susan Mathews, ENT, Dr. Shalini Anandan, Microbiology, Dr. Anna B Pulimood, Pathology, Dr. Mandeep Singh Bindra, Pathology, Dr.Rupa Vedantam, ENT, Unit 3, Dr. Anand Job, ENT, Unit-1, Dr. George Ani Mathew, ENT.

Ref: IRB Min. No. 8449 [OBSERVE] dated 10.09.2013

Dear Dr. Neenu Anna Joseph,

I enclose the following documents:

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

DR. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

CC: Dr. Suma Susan Mathews, ENT, CMC.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to my guide Dr. Suma Susan Mathews, Professor, Department of Otorhinolaryngology, Christian Medical College and Hospital, Vellore for all her hard work, wisdom, expert guidance and encouragement throughout the work on my dissertation. There is not a single area in this thesis where her hands or mind have not touched.

I am grateful to Dr. John Mathew, Professor and Head of Otorhinolaryngology, Christian Medical College and Hospital, Vellore for his support and encouragement in carrying out this study.

I would like to thank Dr. Shalini Anandan and Dr. Balaji Veeraraghavan from the Department of Microbiology for allowing me to conduct this study in collaboration with Microbiology department. I am extremely thankful to Miss. Yamuna, Research Officer from the Department of Microbiology for her efficient contribution in sample processing and interpretation of results.

I am grateful to Dr. Anna B. Pulimood and Dr. Mandeep Singh Bindra from the Department of Pathology, Christian Medical College and Hospital, Vellore for their persistent contribution towards the study.

I am extremely thankful to my co-investigators Dr. Anand Job, Dr. Rupa Vedantam, Dr. George Ani Mathew from the Department of Otorhinolaryngology for their expert advice

and guidance. I am thankful to our PG co-ordinator, Dr. Lalee Varghese for conducting interim thesis update presentations and for helping me to complete the project on time.

I would like to thank Dr.Visali from the Department of Biostatistics for patiently understanding and helping me with the analysis of the data.

I am grateful to all my friends and colleagues from the Department of Otorhinolaryngology for helping me in collecting the samples and making the study a reality. I owe my gratitude to all the endoscopy room staff and theatre staff who helped me in completing the study and to all the patients who consented to be a part of this study. I would also like to thank the Fluid Research Committee, CMC Hospital for granting me permission for conducting this study. A special thanks to my family, especially my husband Dr. Yeldho for the concern and support throughout the work on this study.

And to the Lord Almighty for all his blessings, throughout my life.

CONTENTS

INTRODUCTION

Helicobacter pylori (*H. pylori)* is one of the most common infectious agent found in gastrointestinal tract worldwide (1). *H. pylori* was discovered by Barry Marshall and Robin Warren in 1982. They were awarded Nobel prize in Physiology and Medicine in 2005 for their important discovery that this bacterium was the responsible agent for chronic peptic ulcer. *H. pylori* has got a special tropism for gastric mucosa and is responsible for a variety of gastric diseases ranging from peptic ulcer to gastric adenoma and lymphoma.

Recently, *Helicobacter pylori* has been associated with numerous extra gastric diseases as well. It has been linked to diseases involving cardiovascular, hepatobiliary, dermatological, immunological and haematological systems. The organism has been demonstrated in patients with ischemic heart disease, idiopathic thrombocytopenic purpura, iron deficiency anaemia, colorectal cancers etc (1). *H. pylori* has been detected in saliva, dental plaques and palatine tonsils by polymerase chain reactions. The infection is acquired by oral route (2).

Studies have detected the presence of *H. pylori* in the oral cavity of patients with dyspeptic symptoms (3). Larynx is a region that could be directly exposed to the bacterium by means of laryngopharyngeal reflux. Laryngopharyngeal reflux presents in a variety of manifestations like globus sensation, hoarseness, dysphagia, sore throat, postnasal drip, chronic cough, frequent throat clearing, voice fatigue etc. This has been also associated with multiple laryngeal pathologies like vocal granuloma, polyps, nodules, Reinke's oedema etc. Whether the bacteria reaches the larynx from the stomach by reflux or it reaches directly from the oral

cavity are controversial issues. There are conflicting studies regarding the presence of *H. pylori* in larynx.

Some studies have shown a positive association between *H. pylori* and laryngeal disorders whereas some do not support this. In 2011 Pajić-Penavić et al (4) conducted PCR analysis on cytobrush samples obtained from normal laryngeal mucosa and concluded that *H. pylori* was not present in normal laryngeal mucosa. Previous studies conducted in this field to establish a link between *H. pylori* and laryngeal disorders have yielded conflicting results. The goal of our study was to find the prevalence of *H. pylori* in patients with laryngeal pathologies and the significance of laryngopharyngeal reflux in these patients.

AIM

To study the prevalence of *Helicobacter pylori* in patients with laryngeal pathologies.

OBJECTIVE

Primary objective

To study the infection rate of *Helicobacter pylori* in patients with laryngeal pathologies.

Secondary objective

1) To study the association of laryngopharyngeal reflux in patients with laryngeal pathologies by means of Reflux Symptom Index and Reflux Finding Score.

2) To study the association of laryngopharyngeal reflux in patients whose biopsies are positive for *H. pylori.*

REVIEW OF LITERATURE

Helicobacter pylori infection has been reported throughout the world and is responsible for producing chronic bacterial infection in humans. The prevalence of *H. pylori* largely depends on the standard of living in the region (5). About 50% of global population is thought to be colonised with *H. pylori* and the infection is mostly acquired within 5 years of age. The prevalence varies according to the age, geographic location and the socioeconomic status. The prevalence is much higher in developing countries and this can be attributed to the poor hygiene and overcrowding in these region (6). The prevalence also increases with age and is therefore responsible for development of chronic mucosal inflammation (4). This bacterium has got a high tropism for the acidic environment in the stomach and colonizes the mucosa of the stomach . Once acquired, the infection persists lifelong. Recently the prevalence of *H. pylori* in extra gastric tissues is widely being studied. Fellman et al (7) studied the prevalence of *H. pylori* in the upper aerodigestive tract in patients with proven gastric colonisation and concluded that the bacterium was found in 38% of samples obtained from oral cavity, larynx and pharynx.

Helicobacter pylori is a gram negative micro aerophilic spiral or helical shaped bacteria colonizing human gastric epithelial cells. This bacterium has a tuft of sheathed unipolar flagella which distinguishes it from campylobacters which has got unsheathed flagella. The acidic pH in the stomach is not favourable for its growth and hence they penetrate into the mucous layer of stomach which is resistant to the penetration of acid. The normal gastric pH is about 1-2 whereas it is about 7.4 inside the mucous layer. Optimal growth of *Helicobacter pylori* occurs in presence of 5-10% oxygen. Optimal temperature for its growth is 35^0 C to 37^0C (8).

The gastric antrum is the most favoured site for colonisation by the bacterium. They do not invade the gastric mucosa but are found in the mucus overlying the gastric mucosa. Gastric acid is potentially destructive to the bacteria but protection is provided by the urease generated by them, which converts urea to ammonia thereby neutralising the acid and creating an environment favourable to the bacteria (9).

H. pylori **Secretome.**

The proteins that are secreted by the bacterium are generally grouped under the name "secretome". These proteins play a very important role in pathogenesis, as secreted proteins or those that are present on the external surface of the bacterium are responsible for all the interactions with host. *H. pylori* genome is very small consisting of less than 1600 genes. The bacterium uses a set of secreted and translocated proteins by which it adapts itself to the mucosal environment. The proteins secreted by the bacteria are grouped into different classes namely enzymes, outer membrane proteins, flagellar proteins, binding and transport proteins. Out of the secreted proteins, cytotoxic- associated genes pathogenicity island (*cag* – PAI) plays a very important role in *H. pylori* virulence (10).

The *cag*-PAI is a genomic insert of 27-28 genes. These are present in all type 1 strains of *H. pylori* and are absent in type 2 strains. The *cag*-PAI encodes an effector protein, Cag A. The proteins coded by *cag*-PAI are labelled by the suffix Cag. They are named as Cag A, CagB… CagZ, Cagα, *etc.*, or by a number from 1-28. The *cag*-PAI induces the production of Interleukin 8 (IL-8) by the host which is responsible for triggering inflammatory responses (11). The Vacuolating cytotoxin A, VacA (HP0289) protein is one another major exotoxin secreted by *H. pylori*. This protein has got cytotoxic property by inducing the formation of

intracellular vacuoles thereby producing osmotic swelling of the endocytic compartment and are generally known as pore forming toxins (12). Genes encoding for Cag A and Vac A are present only in type 1 strains of *H. pylori* and hence they are responsible for more severe disease. The bacteria also secretes a variety of enzymes with antioxidant properties like superoxide dismutase (SOD), catalase, thioredoxins and peroxiredoxins, NADPH quinone reductase, and others like proteases, urease etc. Urease enzyme is made up of two subunits namely *UreA* and *UreB.* This enzyme has got buffering activity and acts by splitting urea into ammonia and carbon dioxide thereby neutralising the acid.

Fig .1 *Helicobacter pylori*

Helicobacter pylori is associated with various pathologies like peptic ulcer, non-ulcer dyspepsia and gastric cancer and causes chronic bacterial infections in humans. In human stomach *H. pylori* produces antral predominant gastritis which is linked to duodenal ulceration. This bacterium is also considered as a risk factor for Mucosa Associated Lymphoid Tissue (MALT) lymphoma.

Earlier human stomach was considered as the only reservoir for this bacterium. However recent studies have shown that they can also exist in extragastric tissues like gingiva, human dental plaque, saliva, laryngeal and pharyngeal tissues (1). In a study conducted by Fang et al (13) in patients with hoarseness, *H. pylori* colonisation was found in 13 out of 53 patients with vocal cord polyps by means of Rapid urease testing. Wang et al (14) reported *H. pylori* to be present in the dental plaques and that dental biofilms provided a microaerophilic environment and urea required for the survival of the bacteria.

Clinical spectrum of *H. pylori* infection

H. pylori infection is considered as the main cause of chronic gastritis, peptic ulcer disease and gastric cancer including MALT lymphoma. The bacterial colonisation is usually acquired in childhood and the disease manifestation occurs commonly in adults. Not all of them who are infected manifest the disease and is dependent on the bacterial, host and environmental risk factors. Once *H. pylori* colonises gastric mucosa it induces histologic gastritis with neutrophilic and mononuclear infiltration both in the antrum and corpus of human stomach. However, only a few individuals manifest clinical signs of colonisation in the form of acute gastritis or chronic gastritis or atrophic gastritis with intestinal metaplasia. The life time risk for peptic ulcer disease in *H. pylori* positive individuals is 3-10 times higher than

those who are *H. pylori* negative. *H. pylori* is also found to be responsible for non ulcer dyspepsia or functional dyspepsia (15).

The International Agency for Research on Cancer, a branch of WHO has classified *H. pylori* as a class 1 carcinogen. It has been recognised as a predisposing agent for gastric cancer and gastric marginal zone lymphoma. The most important factor in carcinogenesis is the induction of chronic inflammation by *H. pylori*, there by producing oxygen free radicals leading to DNA damage. The host's immune system plays a critical role in the initiation and progression of infection. The neutrophil activating protein of *H. pylori* stimulates production of IL-12 and IL-23 from neutrophils and macrophages by polarizing T-helper 1 cells (Th 1). Also Th1 cytokines like gamma interferon (IFN- γ) and tumour necrosis factor alpha (TNF- α) increases the release of pro inflammatory cytokines and augment the apoptosis caused by *H. pylori* (16).

Association between gastroesophageal reflux disease and *Helicobacter pylori* positivity have yielded conflicting results. Gastroesophageal reflux disease and its complications like Barrett's oesophagus and oesophageal cancer has shown an inverse relationship with *H. pylori* according to some studies. Most studies have shown a probable protective role of *H. pylori* against GERD. Physiology of gastric acid production depends on the gastric location of the bacteria (17). Infection of the gastric corpus by the bacteria causes atrophy of the gastric parietal cells which are responsible for acid production, resulting in hypochlorhydria and decrease of reflux. Infection of gastric antrum leads to destruction of somatostatin secreting cells present in the gastric antrum. Somatostatin secreted by the D cells of the gastric antrum plays a negative feedback in acid production. Studies have shown that destruction of these cells leads to increase acid production and there by increased reflux (18). Polat et al (19) studied the severity of GERD and *H. pylori* positivity in patients with GERD and concluded that the bacterium was present in 82.5% of patients with GERD.

Detection of *H. pylori.*

There are different methods to detect *Helicobacter pylori.* It can be diagnosed by invasive and non invasive methods. Non invasive tests include Urea breath test, serology and whole blood antibody tests whereas invasive tests involves taking tissue endoscopically for biopsy and further studies.

Urea Breath test (Carbon isotope Urea Breath Test)

The patient should be advised not to take any antibiotics, bismuth containing medications or proton pump inhibitors up to two weeks prior to the test. The patient is asked to swallow a compound containing urea labelled with ^{13}C. In the presence of *H. pylori*, the urea is converted by the urease present in the bacterium into carbon dioxide which is traced in the exhaled air after 10 minutes. This test can also be used after treatment to confirm that all *H. pylori* has been eradicated (20).

Blood tests

These tests measures *H. pylori* IgG and *H. pylori* cagA IgG antibodies in the blood. If the test is positive, it indicates that the patient was infected by the organism but does not tell that the infection is acute or chronic. The test can remain positive for several years even after the infection is treated and patient is cured.

Biopsy

The most accurate method of detecting *H. pylori* is by studying a tissue sample obtained by biopsy. Various tests can be done on the biopsied sample to detect the bacterium. These include Rapid urease testing, Giemsa staining, immunohistochemical staining, culture, and Polymerase Chain Reaction. *H. pylori* is said to have the maximum urease activity of any bacterium known to infect man (21).

In rapid urease testing (RUT), one or two pieces of tissue are placed in an agar well containing urea and a pH reagent. Urease present in the bacterium cleaves urea to liberate ammonia resulting in an alkaline pH change and a subsequent colour change. The urease tests will give false negative results in subjects who have been on proton pump inhibitors, H2 receptor antagonists, antibiotics and also in patients with recent history of gastrointestinal bleed. The presence of other urease producing organisms will give false positive results. Sensitivity of RUT ranges 80% to 90% depending on the test used and the condition under which the test was performed.

The histopathological diagnosis of *H. pylori* is widely employed in gastric biopsy specimens. Presence of features of chronic inflammation in the histology is the hallmark of active *H. pylori* infection (22). Stains used in the diagnosis include modified Giemsa, Warthin-Starry stain, Gimenez, Genta and immunohistochemical stains. The sensitivity with routine haematoxylin eosin staining is low especially when there are not many bacteria. With giemsa staining *H. pylori* appears as a blue or grey rod like structure in a pink or pale blue background with a blue nuclei. Giemsa staining is sensitive, cheap, easy to perform, and reproducible. One of the disadvantages of Giemsa staining is that there is

little contrast between the organism and the surrounding tissue. Immunohistochemical staining techniques overcomes this. Immunohistochemical stain is considered to be the gold standard in histological diagnosis, as this a highly sensitive and specific test (23).

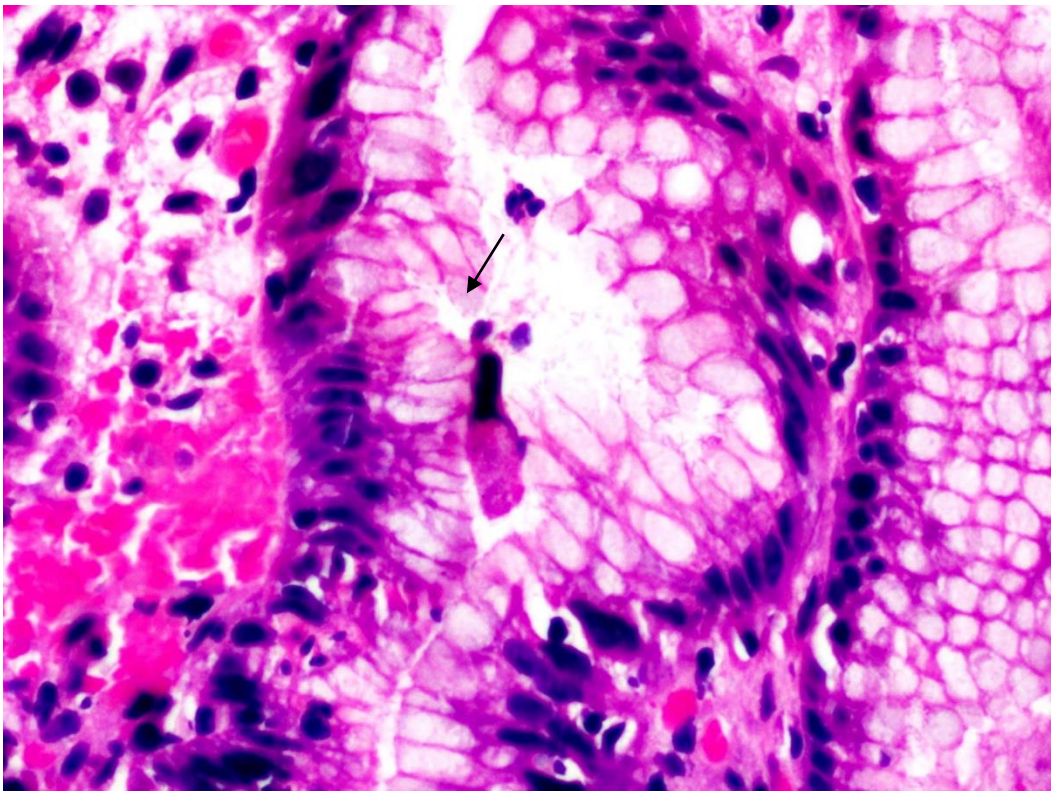


Fig. 2 Haemotoxylin and Eosin staining for *H. pylori* in stomach

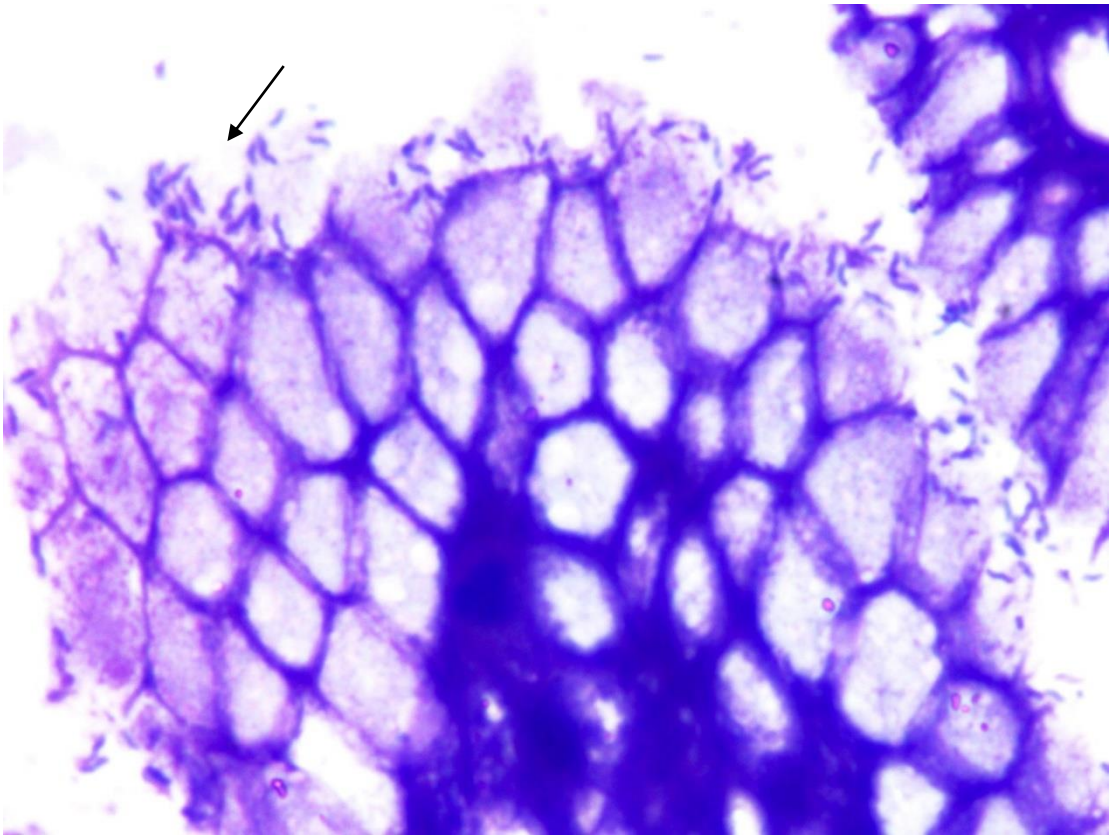


Fig. 3 Giemsa staining for *H. pylori* in the stomach

Studies have shown that Polymerase Chain Reaction (PCR) is the best method for detection of *H. pylori* and could detect bacteria in those samples which other methods could not detect. In PCR, specific sequences within a DNA can be identified which are amplified to many thousand to million folds using sequence specific oligonucleotides, heat stable DNA polymerase and thermal cycling.

In PCR, a special DNA polymerase obtained from *Thermophilus aquaticus* (Taq polymerase) is used to make many copies of a short length of DNA. The PCR amplifies DNA exponentially doubling the number of target molecules with each amplification. By using PCR, genes from pathogens can be identified and amplified. Good primer design is one of the most important parameters in real time PCR. Primers should be 18-24 nucleotides in length. Real time PCR differs from traditional PCR in that amplified product is measured at the end of each cycle (24). Real time PCR has got many advantages over conventional PCR such as high specificity, short work time and low risk of contamination.

In a study conducted by Titiz et al (25) , PCR testing was done for detection of *H. pylori* in patients with laryngeal pathologies. The PCR results of 17 out of 21 samples of patients with laryngeal squamous cell carcinoma was positive for *H. pylori* and no genomic material of *H. pylori* was found in patients with benign laryngeal pathologies. In one other study conducted by Ozyurt et al (26) *Helicobacter pylori* virulence factor cag A was detected in 17 out of 29 cases of laryngeal biopsy specimen which included both benign and malignant lesions by PCR analysis.

Treatment of *H. pylori* infection.

H. pylori infection is very common in general population, but it is not necessary that all of them who are infected needs to be treated (21). Treatment is indicated for patients with peptic ulcer disease or with gastric MALT lymphoma. Combination drug regimens maximizes the chance of eradication and minimize the development of drug resistance. The most widely accepted regimen in the past was the standard triple therapy which included a proton pump inhibitor (PPI), clarithromycin, and amoxicillin or

metronidazole for a period of at least 7 days. But clarithromycin resistance became one of the major reasons for failure of eradication of *H. pylori.*

Newer regimens like sequential therapy, concomitant therapy and hybrid therapy came into picture to overcome this. Sequential therapy is basically a dual regimen and consisted of a five day course of PPI with amoxicillin followed by a five day course of PPI with two other antibiotics, usually clarithromycin and metronidazole. Sequential therapy was formulated in the year 2000 in response to failure of triple therapy, but eventually both clarithromycin and metronidazole resistant strains evolved.

In concomitant therapy, the standard triple therapy is converted to quadruple therapy by addition of 500 mg of metronidazole or tinidazole twice daily. The drugs are given for a short duration of 5 days. This, one PPI and three antibiotic regimen showed more success rates than the previous regimens (27). For patients with failure of concomitant regimen, *H. pylori* rescue regimen with levofloxacin was employed and has shown promising results. This consists of triple therapy with PPI, levofloxacin and amoxicillin for a period of 10 days (28).

ANATOMY OF LARYNX

Larynx is a dynamic organ that is responsible for maintaining several functions like respiration, swallowing and phonation. Larynx is made up of four anatomic units: laryngeal framework, mucosa, intrinsic and extrinsic muscles. A supporting skeleton made up of cartilages and intervening membranes is responsible for maintaining its shape. Larynx extends from the laryngeal inlet to the inferior border of the cricoid cartilage. The laryngeal framework is made up of three unpaired cartilages namely epiglottis, thyroid and

cricoid and three paired cartilages namely arytenoid, corniculate and cuneiform cartilages (29).

Epiglottis

Epiglottis is a flexible, leaf shaped form of elastic cartilage which forms part of laryngeal inlet. Inferiorly epiglottis tapers to a point called petiole which is attached to the thyroid cartilage just above the anterior commissure. The hyoid bone divides the epiglottis into suprahyoid and infrahyoid portions. The suprahyoid portion projects behind the base of tongue and thereby protects the larynx during swallowing (30).

Thyroid cartilage

Thyroid cartilage is composed of two laminae which are fused in midline and spans the entire length of true and false vocal folds. They form a variable angle approximately 75 to 90 degrees in adult male and 90 to 110 degrees in adult female. The thyrohyoid ligament attaches to the superior border of the lamina and the cricothyroid ligament attaches to the inferior border on the medial aspect. The two laminae fuse in the midline anteriorly to form the laryngeal prominence. The thyroid cartilage is lined by a thick layer of perichondrium all around except at the region of anterior commissure. Five ligaments namely the median thyroepiglottic ligament, the two vestibular ligaments and the two vocal ligaments are attached here from superior to inferior. This attachment forms the anterior commissure tendon or the Broyles' tendon which constitutes an important barrier to the spread of laryngeal neoplasms (31).

Cricoid cartilage

The cricoid cartilage forms a complete cartilaginous ring of the airway and is taller posteriorly forming most of the posterior wall of larynx. Cricoid cartilage serves as the most important structural support for a functioning larynx since it is a complete ring. The posterior lamina is broader than the anterior and the inferior border is flat. The posterior lamina extends cranially to articulate with the arytenoids forming the cricoarytenoid joints which are crucial joints for voice production. The inferior border is attached to the first tracheal ring via the cricotracheal ligament. Since this is the only cartilage that forms a complete ring surrounding the airway, it is more prone to injury and is a common site for airway narrowing (32).

Arytenoid cartilage

These are paired, three sided pyramids with an anterior process (vocal process) and a lateral process (muscular process) and an apex and a base. The vocal process attaches to the vocal ligament and the muscular process attaches to the posterior cricoarytenoid muscle. The medial surface forms the lateral boundary of the posterior glottis. Arytenoids are capable of rocking, gliding and rotatory actions around the cricoarytenoid joint. Sitting atop the arytenoid cartilage is the corniculate cartilage.

Corniculate and Cuneiform cartilages

They are paired elastic fibrocartilages. Corniculate cartilages (Cartilage of Santorini) are located in the posterior part of aryepiglottic fold and they articulate with the

apex of arytenoid cartilage through synovial joints. Cuneiform cartilages (Cartilage of Wrisberg) are located in the margin of aryepiglottic folds adding rigidity to these folds.

Ligaments of larynx

The connective tissue of the larynx condenses to form extrinsic and intrinsic ligaments. The extrinsic ligaments connect the larynx to the surrounding structures whereas the intrinsic ligaments connect the laryngeal cartilages to each other.

The thyrohyoid ligament, the hyoepiglottic ligament, the glossoepiglottic ligament and the cricotracheal ligament are the extrinsic ligaments of larynx. The intrinsic ligament defines the skeletal outline of larynx by maintaining the nine cartilages in position. These are the quadrangular membrane, conus elasticus and thyroepiglottic ligament.

The quadrangular membrane lies between the lateral surface of epiglottis anteriorly, the medial surface of arytenoid cartilage posteriorly reaching up to the ventricular ligament inferiorly. The superior margin of this forms the aryepiglottic fold. Conus elasticus arises from the superior border of cricoid cartilage and attaches on the lower anterior border of thyroid cartilage forming the cricothyroid membrane. The superior aspect forms the vocal ligament. In the subglottis the conus elasticus lies in the submucosal plane (32).

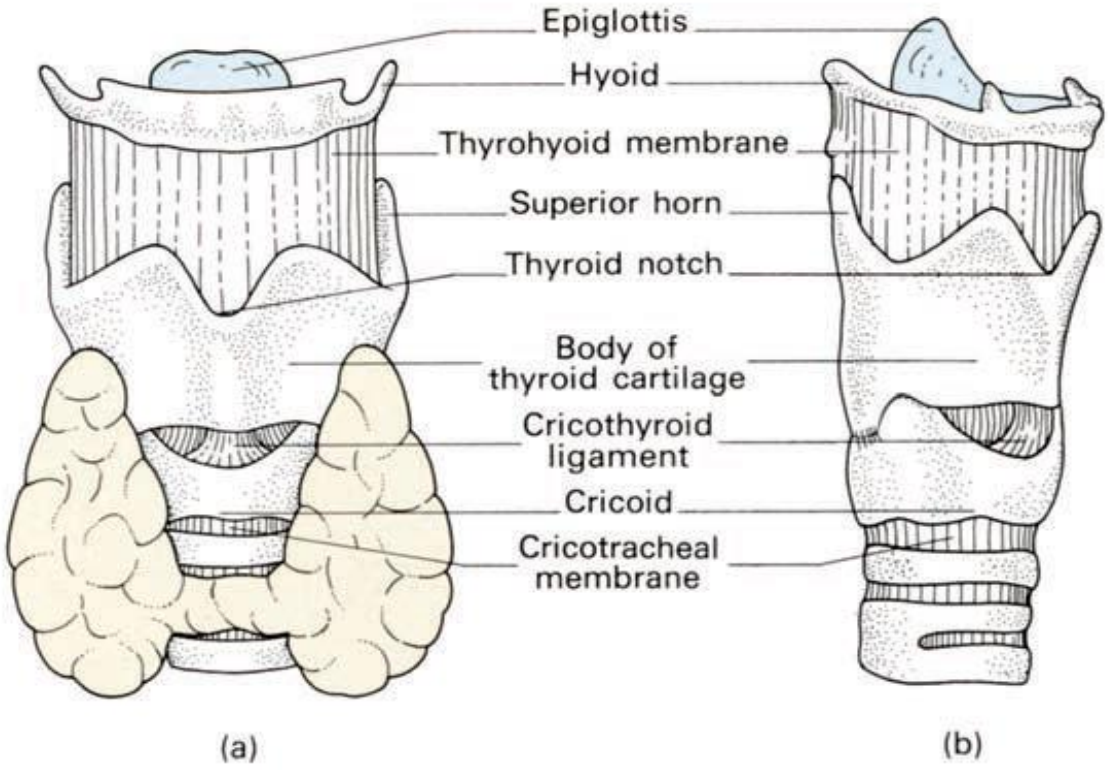


Fig. 4 External framework of larynx (*adopted from the book Clinical Anatomy by Harold Ellis*)

Muscles of Larynx

Muscles of larynx are divided into intrinsic and extrinsic muscles. Extrinsic muscles are responsible for maintaining the position of larynx in the neck whereas intrinsic muscles are responsible for maintaining the vocal fold shape and movement.

The intrinsic muscles are connected to the laryngeal cartilages. The intrinsic muscles are all paired and move the cartilages of larynx thereby regulating the mechanical properties of the larynx. The adductors of vocal cord are the lateral cricoarytenoid, thyroarytenoid and the interarytenoid muscles.

The lateral cricoarytenoid arises from the superior border of lateral part of arch of cricoid cartilage and inserts on to the muscular process of the arytenoid. They adducts the vocal fold thereby making its edge sharp and passively stiffened.

The thyroarytenoid arises from the inner thyroid cartilage and inserts on to the vocal process of the arytenoid and anterolateral surface of body of arytenoid. This is mainly concerned with maintaining the tension of vocal folds. The medial belly of thyroarytenoid is known as the vocalis muscle and it forms the body of the vocal fold. The medial compartment of thyroarytenoid muscle has a higher concentration of slow twitch muscle fibres whereas the lateral compartment has higher concentration of fast twitch muscle fibres. Thus medial compartment is specialised for phonation whereas lateral compartment is specialised for vocal fold adduction (33).

The posterior cricoarytenoid is the sole muscle which abducts or opens up the vocal folds. It originates from the posterior surface of the cricoid cartilage and inserts on to the muscular process of the arytenoid (34).

Cricothyroid arises from the lateral surface of anterior arch of cricoid cartilage and inserts laterally onto the anterior border of inferior cornu of thyroid cartilage and posterior part of lower border of thyroid lamina. Cricothyroid muscle stretches the vocal cords and is an important muscle for professional singers.

Extrinsic laryngeal muscles are primarily strap muscles which maintain the position of larynx in neck. They help the intrinsic musculature to work effectively by maintaining a stable laryngeal skeleton. The extrinsic muscles are divided into infrahyoid and suprahyoid muscles. The infrahyoid muscles are sternohyoid, thyrohyoid, sternothyroid and omohyoid. The suprahyoid muscles are digastric, mylohyoid, geniohyoid and stylohyoid.

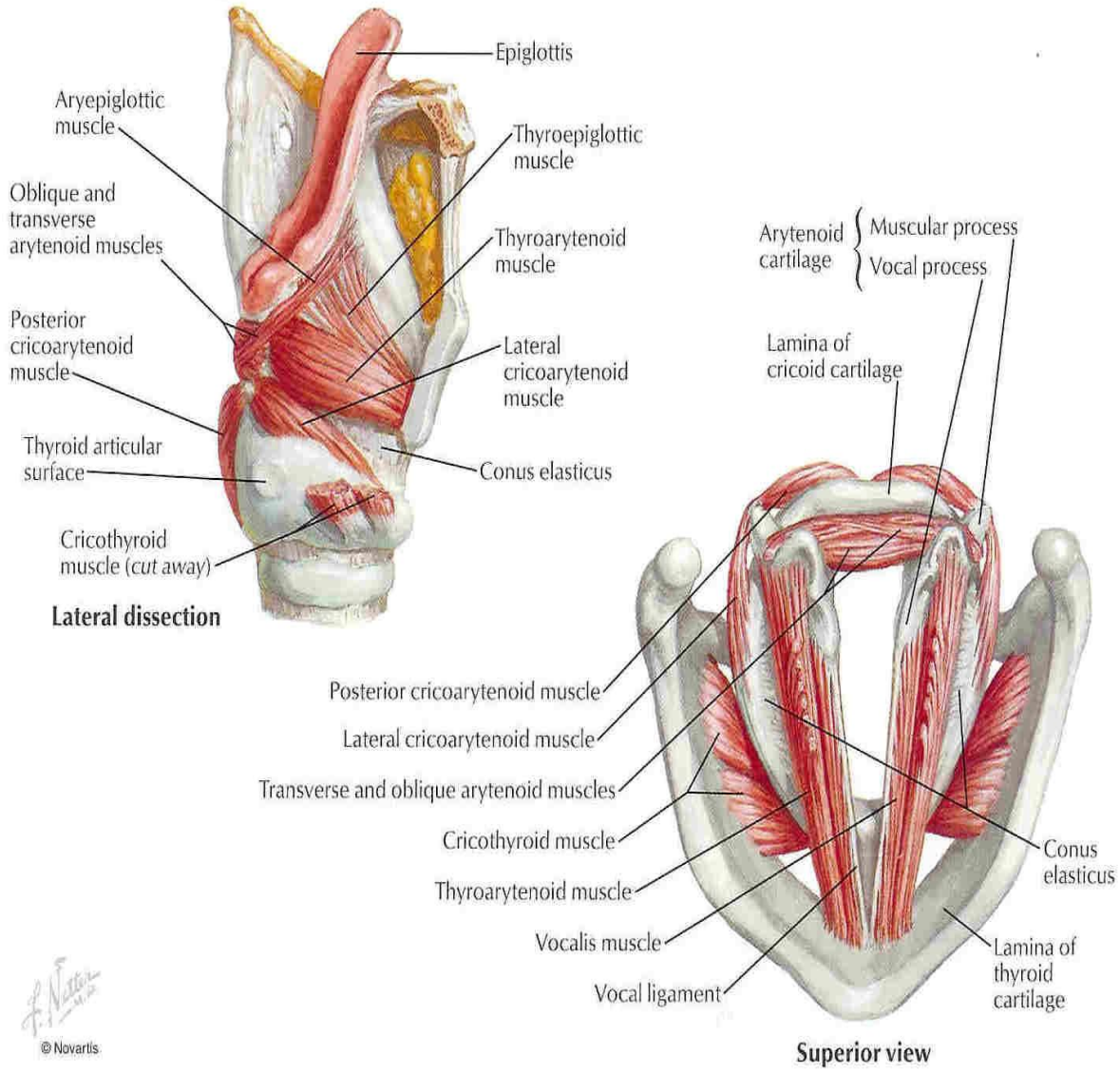


Fig. 5 Intrinsic muscles of larynx. (*Adopted from Netter's Colour Atlas*)

All intrinsic muscles of larynx are innervated by the recurrent laryngeal nerve except for the cricothyroid which is innervated by the superior laryngeal nerve. Recurrent laryngeal nerve and superior laryngeal nerve are the branches of the tenth cranial nerve or the Vagus nerve. The superior laryngeal nerve branches off the vagus nerve at the inferior end of nodose ganglion and divides into internal and external laryngeal nerve near the greater cornu of hyoid bone. The external branch supplies the cricothyroid muscle and the internal

branch is responsible for innervation of laryngeal mucosa above the level of vocal fold. The internal branch enters the larynx after piercing the thyrohyoid membrane along with the superior laryngeal artery (34).

The recurrent laryngeal nerve branches off the vagus in the upper thorax and has got different course on the right and left sides. The right recurrent laryngeal nerve branches off the vagus near the right subclavian artery and then travels in the right tracheoesophageal groove. The left recurrent laryngeal nerve winds around the aortic arch distal to the ligamentum arteriosum, and then enters the tracheoesophageal groove in the chest. The right nerve loops around the right subclavian artery and both of them travel superiorly towards the larynx. Thus the left has a longer course than the right and is more vulnerable to injury. Both the nerves enter the larynx behind the cricothyroid joint deep to the fibres of inferior constrictors (32). There are also interconnections between the superior and recurrent laryngeal nerves, particularly in the interarytenoid muscle (33).

The arterial supply of larynx is derived from the laryngeal branches of superior and inferior thyroid arteries and the cricothyroid branch of the superior thyroid artery. The lymphatic drainage of larynx is divided by the vocal folds into upper and lower drainage groups. The larynx above the vocal folds is drained by vessels that accompany the superior laryngeal vein and pierce the thyrohyoid membrane emptying into the upper deep cervical lymph nodes. The larynx below the vocal folds drain into the lower deep cervical chain through the prelaryngeal and pretracheal nodes. The free edge of vocal fold is devoid of lymphatics.

The lymph nodes of the neck were categorised into different levels by the head and neck surgeons at the Memorial Sloan-Kettering Hospital into six different zones or levels on each side (35).

Level I: includes the submental (Ia) and submandibular groups (Ib). The submental group is confined between the boundaries of submental triangle and the submandibular nodes lies in the submandibular triangle.

Level II: includes the upper jugular group located around the upper third of Internal Jugular vein extending from the level of carotid artery bifurcation (surgical landmark) or horizontal plane passing through the inferior border of hyoid bone (clinical landmark) inferiorly to the skull base superiorly, laterally up to the posterior border of sternocleidomastoid and medially up to the lateral border of stylohyoid muscle. Level II node is subdivided into two IIa and IIb by the spinal accessory nerve.

Level III: includes nodes located along the middle third of the internal jugular vein and extends from the carotid artery bifurcation superiorly to the junction of the crossing of the omohyoid muscle with internal jugular vein (surgical landmark) or from the horizontal plane passing through the inferior border of hyoid bone to the inferior border of cricoid cartilage (clinical landmark).

Level IV: these are the lower jugular group and extends from horizontal plane passing through the inferior border of cricoid cartilage superiorly to clavicle inferiorly.

Level V: includes the posterior triangle group.

Level VI: nodes include those lying in the anterior central compartment of neck extending from the level of hyoid bone superiorly to the suprasternal notch inferiorly. The lateral boundaries are the common carotid arteries. The perithyroidal, paratracheal, prelaryngeal (Delphian) nodes and nodes along recurrent laryngeal nerves lie in this group (36).

Recently a Level VII group of lymph nodes is being described. This refers to the extension of paratracheal nodes below the suprasternal notch up to the level of the innominate artery. These are also known as superior mediastinal nodes (37) .

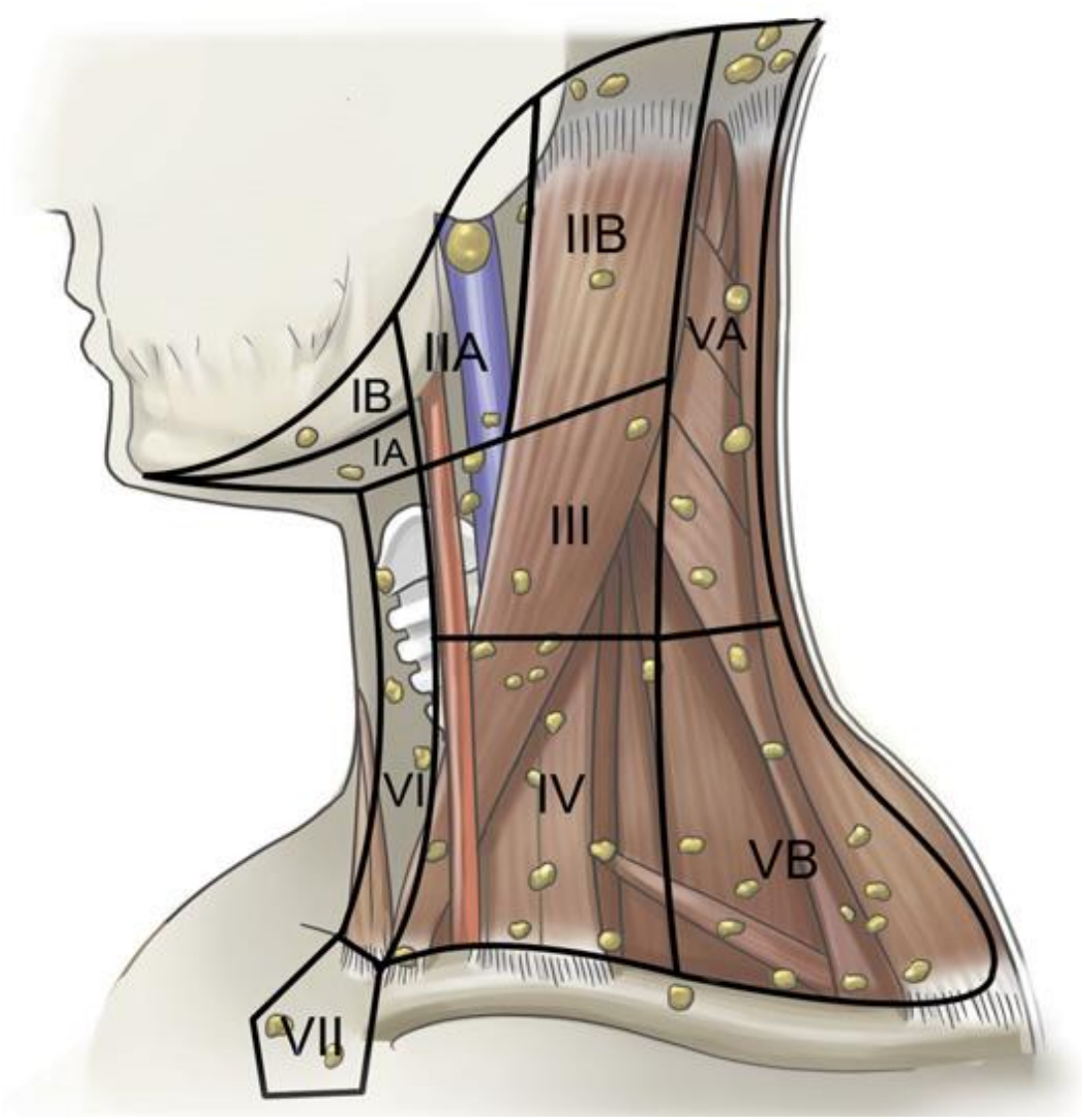


Fig. 6 Cervical lymph nodes divided into seven levels (*Adopted from Otolaryngol Clin N Am 45 (2012) 1363-1383*

Larynx is divided into three regions or sites namely supraglottis, glottis and subglottis. The supraglottis extends superiorly from the tip of the epiglottis to the apex of the ventricles and undersurface of the false cords inferiorly. It includes the both the lingual and laryngeal surface of the epiglottis, the false cords (ventricular bands), the arytenoids and the aryepiglottic folds (laryngeal surfaces only). The inferior limit of supraglottis is a horizontal line passing through the lateral margin of the ventricle at its junction with superior surface of

true vocal cords. Supraglottis has rich lymphatic supply and therefore tumours involving this region has high propensity for cervical lymph node metastasis.

The glottis is composed of true vocal cords and the anterior and posterior commissures. The posterior two fifths of the vocal fold is cartilaginous and the anterior three fifths is membranous. Most of the vibratory function occurs in the membranous portion. The inferior limit of glottis is 1cm inferior to the inferior limit of supraglottis (31). The anterior commissure lies midway between the thyroid notch and lower border of thyroid cartilage in males whereas it lies at the junction of upper one third and lower two thirds in females. Subglottis extends from the inferior limit of glottis to the inferior border of cricoid cartilage.

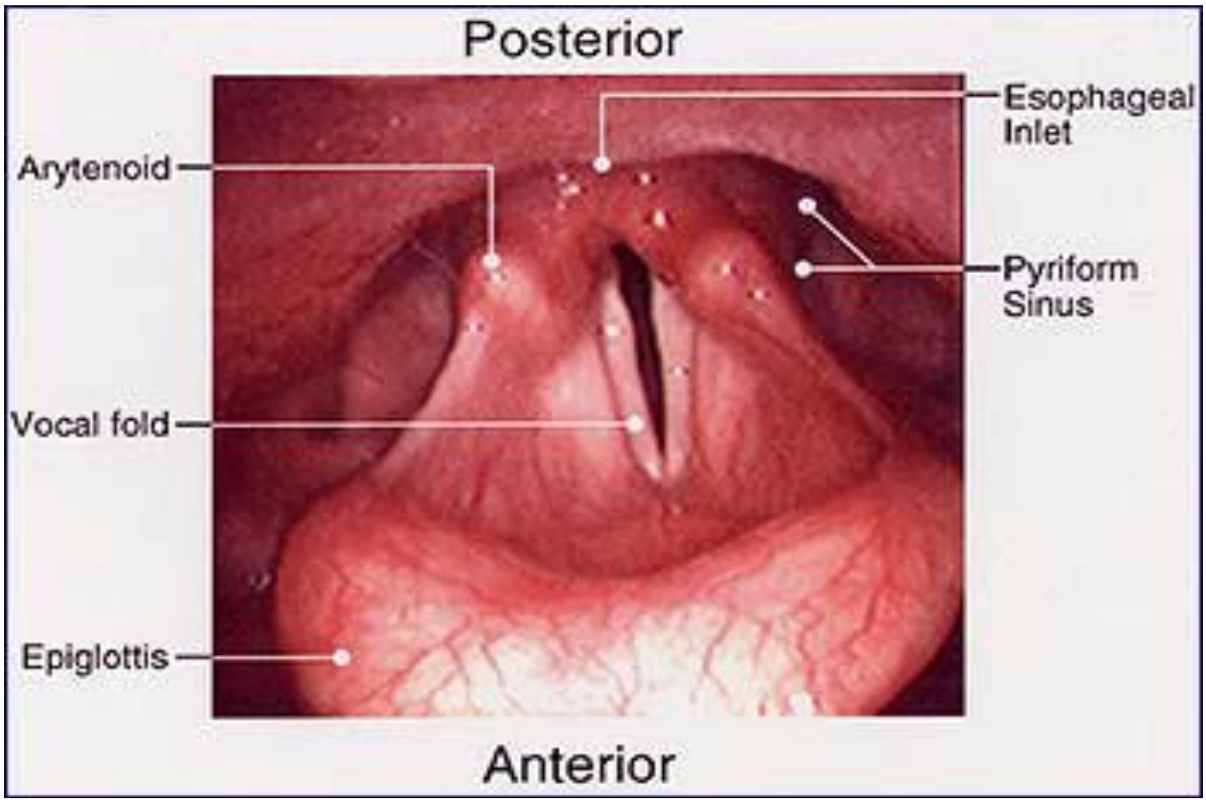


Fig. 7 Normal larynx.

Histology of larynx

The mucosa of larynx is lined predominantly by pseudostratified ciliated columnar epithelium or the respiratory epithelium except the vocal folds, the edges of aryepiglottic folds, the anterior surface and upper portion of posterior surface of epiglottis. These areas are lined by stratified squamous epithelium (32). The true vocal cords are lined by stratified squamous epithelium better suited to withstand the trauma of vocal fold contact. The subglottis is also lined by pseudostratified columnar epithelium.

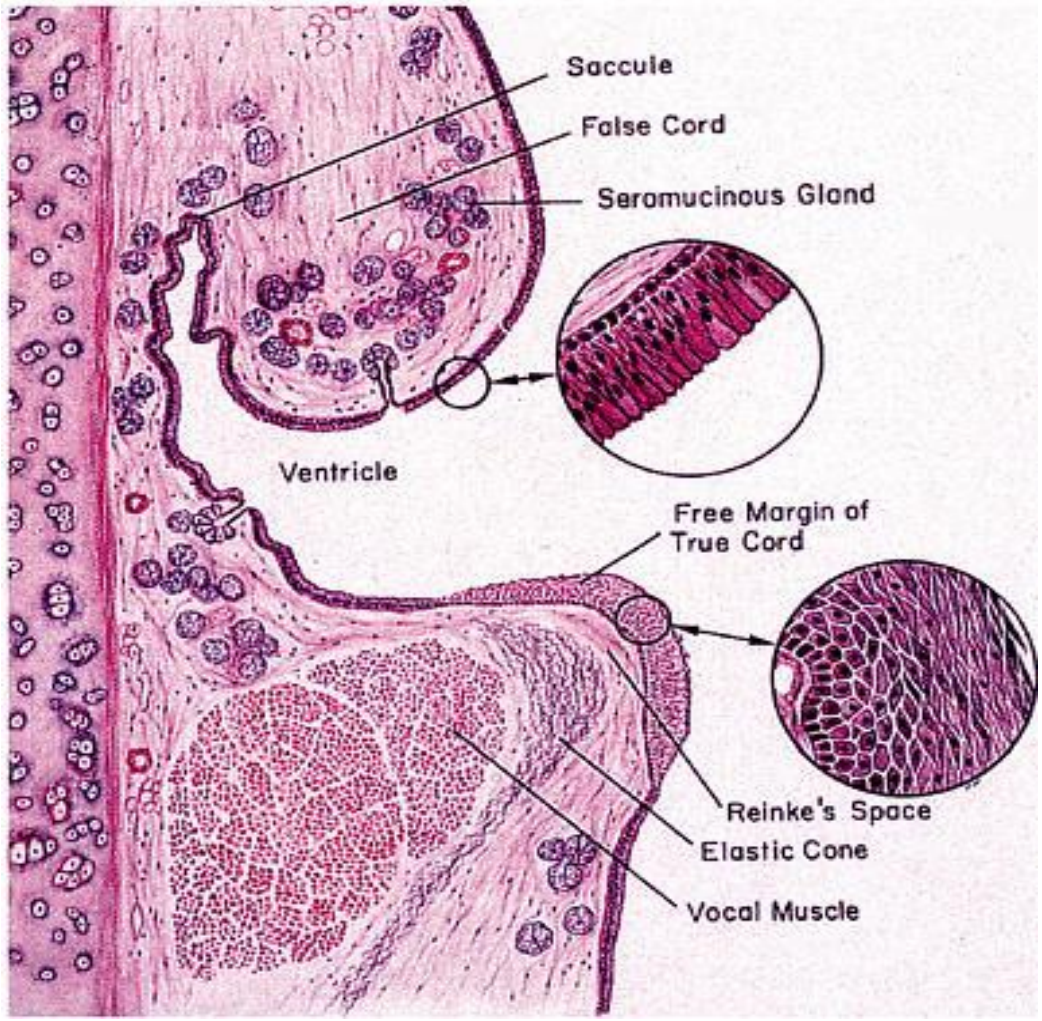


Fig. 8 Histology of laryngeal mucosa (*Adopted from Mills SE, Fechner RE, Pathology of the larynx , Atlas of Head and Neck Pathology Series*)

Microanatomy of the vocal fold

The stucture of vocal fold is very complex and is composed of five layers. The outer epithelial layer acts like a capsule and helps in maintaining the shape of the vocal fold. The vibratory surface of this is lined by stratified squamous epithelium. Beneath the epithelium lies the Reinke's space and the vocal ligament. The superficial layer of lamina propria forms the Reinke's space and it is composed of loose fibrous components and matrix with a few fibroblasts. The intermediate and deep layers form the vocal ligament and lies immediately beneath the Reinke's space. The intermediate layer does not contain fibroblasts and is mainly made of elastic fibres. The deep layer is rich in fibroblasts and is primarily composed of collagenous fibres. This structure is not uniform throughout the entire length of vocal cord. At the anterior end of the vocal cord, the intermediate layer becomes thickened to form an oval mass which is known as anterior macula flava. It inserts anteriorly into the anterior commissure tendon and is made of fibroblasts, stroma and elastic fibres. Similarly at the posterior end, the intermediate layer becomes thickened to form the posterior macula flava, which is attached to the vocal process of the arytenoid cartilage. Both of them acts as cushions that protects the ends of vocal cord from mechanical damage caused by contact or vibrations. Deep to lamina propria lies the thyroarytenoid or the vocalis muscle.

Even though the vocal fold is composed of five layers, mechanically it acts like a three layered structure. The three layers are the cover, transition and the body. The cover is formed by the epithelium and the Reinke's space, the transition by the intermediate and deep layers of the lamina propria and the body by the vocalis muscle. Recent research has shown an additional layer of basement membrane connecting the epithelium to the superficial layer of lamina propria (33) .

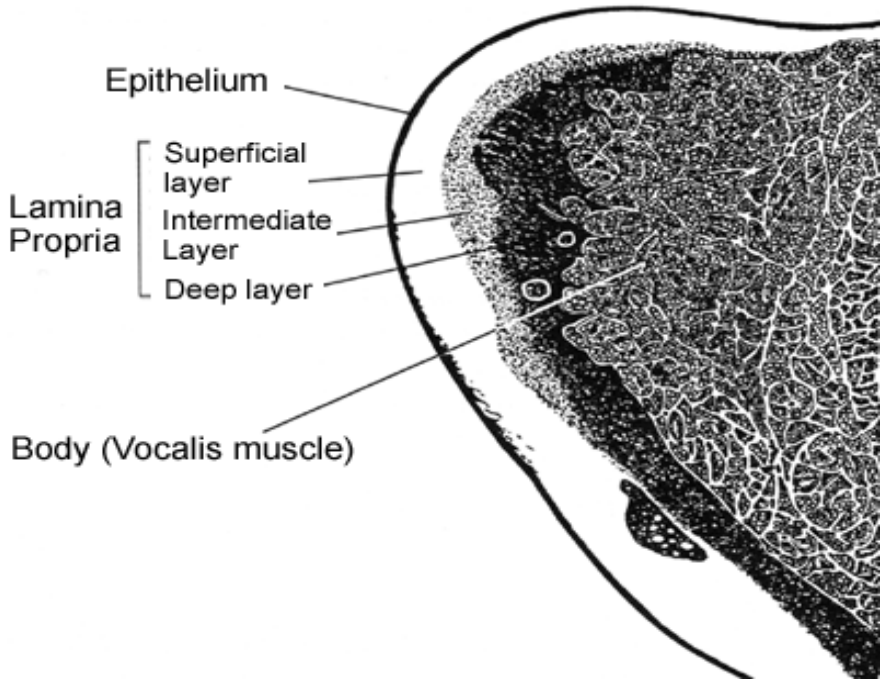


Fig. 9 Microanatomy of vocal cord (*Adopted from Tom Harris et al, The Voice Clinic Handbook, Whurr Publishers, 1998)*

There are two potential spaces of larynx namely the pre epiglottic space and the paraglottic space. The pre epiglottic space is bounded superiorly by the hyoepiglottic ligament, anteriorly by the thyroid cartilage and the thyrohyoid membrane, posteriorly by the epiglottis and the thyroepiglottic ligament. Laterally the pre epiglottic space opens into the paraglottic space. The pre epiglottic space is composed of fat and areolar tissue and is frequently invaded by tumours through the small fenestrations over the posterior surface of epiglottis. The paraglottic space lies lateral to the true and false cords. It is bounded medially by the quadrangular membrane, the ventricle and conus elasticus, laterally by the thyroid cartilage anteriorly and the mucosa of the medial wall of pyriform sinus posteriorly, and inferolaterally by the cricothyroid membrane. The paraglottic space together with the pre epiglottic space forms a horseshoe shaped fatty space around the internal laryngeal structures (29).

BENIGN LARYNGEAL DISORDERS

Voice plays a key role in one's identity and vocal dysfunction has a significant psychosocial impact on one's life. Voice disorders may result from pathologies affecting the vocal cords. These are broadly classified into benign and malignant disorders. Professional voice users like teachers, singers, lawyers etc. are at risk of developing benign vocal cord disorders. Sulkowski et al (38) analysed 1261 cases of occupational voice disorders over a 5 year period and reported that 66% of these patients were primary school teachers and 55% of referrals were at 51- 60 years of age. Benign lesions of vocal cord are noncancerous growth of abnormal tissue occurring in the vocal cords. According to the nomenclature system put forward by Rosen et al (39), they are referred to as Benign Vocal Fold Lesions (BVFLs). They include nine distinct vocal fold lesions.

1) Vocal fold nodules
2) Vocal fold polyps
3) Vocal fold cysts - subepithelial
4) Vocal fold cysts - ligamental
5) Pseudocysts
6) Reactive lesions
7) Nonspecific vocal fold lesions (NSVFL)
8) Vocal fold fibrous mass- subepithelial
9) Vocal fold fibrous mass- subligamental.

Others include intracordal scarring, granulomas, papillomas, Reinke's oedema, vocal cord ectasia and leukoplakia involving the cords. Singhal et al (40) conducted a study to identify the demographic characteristics and symptomatology of 50 cases of benign tumours of larynx and concluded that majority of these tumours occurred in males and 66% of patients had vocal cord polyps with hoarseness of voice being the most prominent presenting symptom.

VOCAL NODULE:

Vocal nodules are defined as bilateral small swellings which are less than 3mm in diameter. These are seen in children, more commonly in boys and in professional voice users, predominantly in women. They are also known as singer's nodes, screamer's nodes or teacher's nodules. Patients present with hoarseness, frequent voice breaks and voice fatigue. They are usually located over the free edge of the vocal cord at the junction of anterior one third and posterior two thirds (29). This is because this is the region where the vocal cords are subjected to maximum shearing and collision forces. The repeated collision leads to formation of localised vascular congestion and oedema and in long run leads to hyalinisation of the Reinke's space with thickening of overlying epithelium and development of epithelial hyperplasia.

The histology of nodule is very important to differentiate it from vocal cord polyps. In vocal nodules, epithelium shows hyperplasia with parakeratosis with thickening of basement membrane and fibrosis of lamina propria (41). Stroboscopy shows an hour glass closure of glottis. Treatment options include medical management with antireflux measures and speech therapy. Surgical option may be considered as a last option for patients who fail several sessions of speech therapy and remain symptomatic. Surgical techniques include microsurgical methods or laser excision.

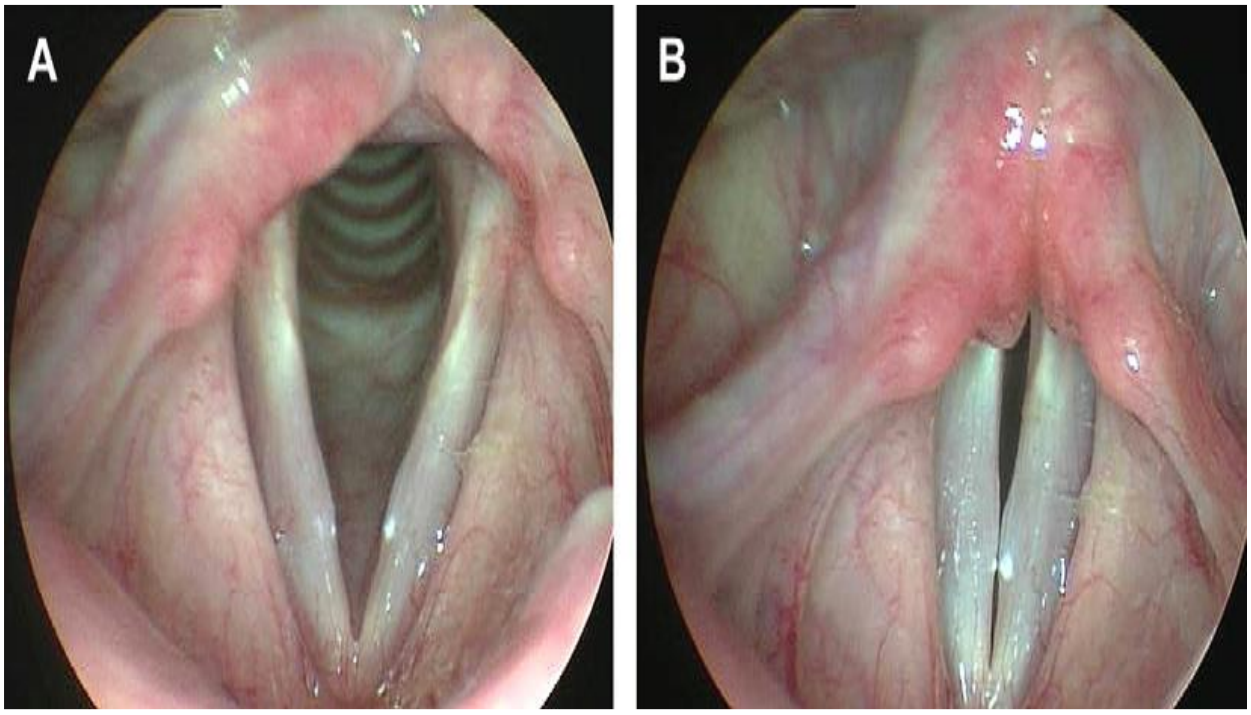


Fig. 10 Vocal fold nodules A. During inspiration B. During Phonation (*Adopted from Otolaryngol Clin N Am40 (2007) 1091–1108)*

VOCAL FOLD POLYPS

Vocal polyp is a benign swelling greater than 3mm arising from the free edge of vocal fold. They affect men more than women and are frequently seen between ages of 30 and 50 years. Aetiological factors include intense vocal abuse as yelling or shouting, history of aspirin or anticoagulant use, history of trauma like endotracheal intubation (22). They affect men more than women. Histological features are characterised by breakage of capillaries in the Reinke's space with surrounding oedema and deposition of amorphous

material and hemosiderin. The basement membrane in case of polyps is normal or thinned out. Polyps may be broad based or pedunculated, haemorrhagic or non haemorrhagic. On stroboscopy, small polyps show intact mucosal wave but phase asymmetry due to impaired phase closure and mass effect whereas large polyps show decreased mucosal wave amplitude. Polyps are removed by surgical excision under general anaesthesia followed by speech therapy.

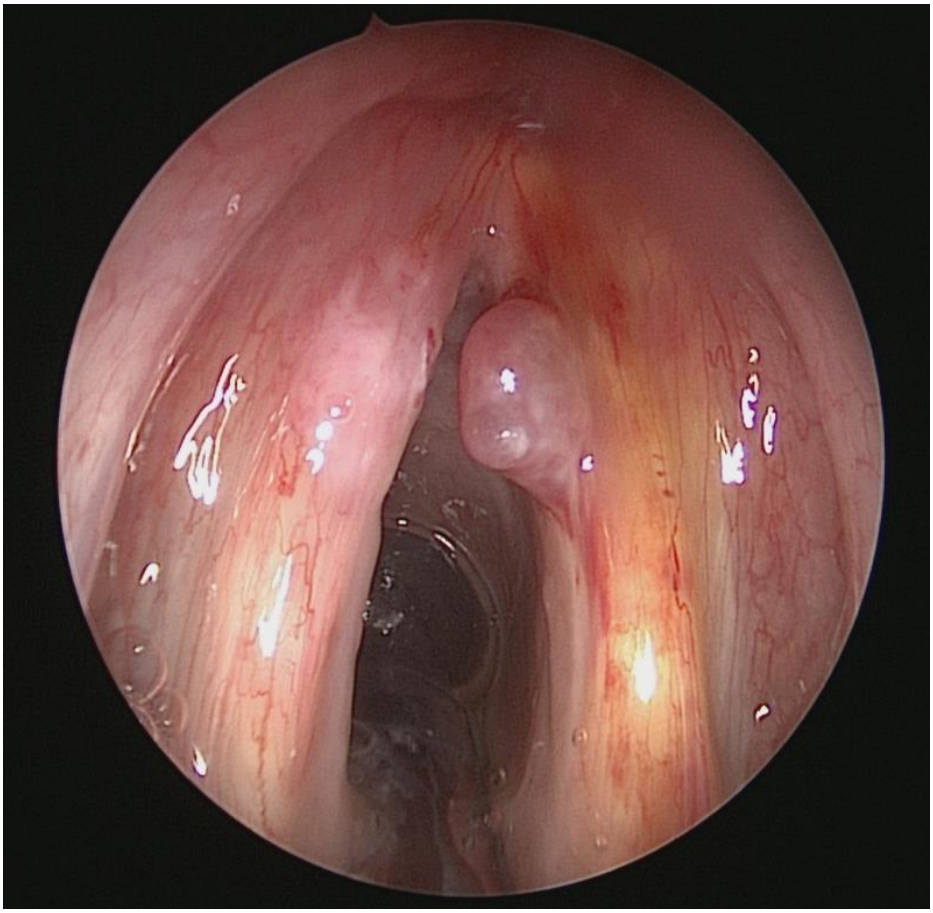


Fig. 11 Right vocal cord polyp

VOCAL CORD CYSTS

Cysts are subepidermal epithelial-lined sacs which are located within the lamina propria. They are of two types: a mucus retention cyst and an epidermoid cyst. Mucus retention cyst develop when the duct of a mucus gland gets blocked, retaining the secretions (38). It is lined by cuboidal or low columnar epithelium. It is usually unilateral and is found on the free edge of the vocal fold (29). Epidermoid cysts may develop from congenital cell rests or as a result of microinclusion of epithelium from surface trauma. These are lined by squamous epithelium and are filled with cholesterol and keratin debris. Stroboscopy reveals asymmetric vocal folds with a subepithelial mass and absent mucosal wave. Sometimes the cyst may rupture spontaneously and if the resultant opening is small as compared to the cyst, it forms an open cyst (31).

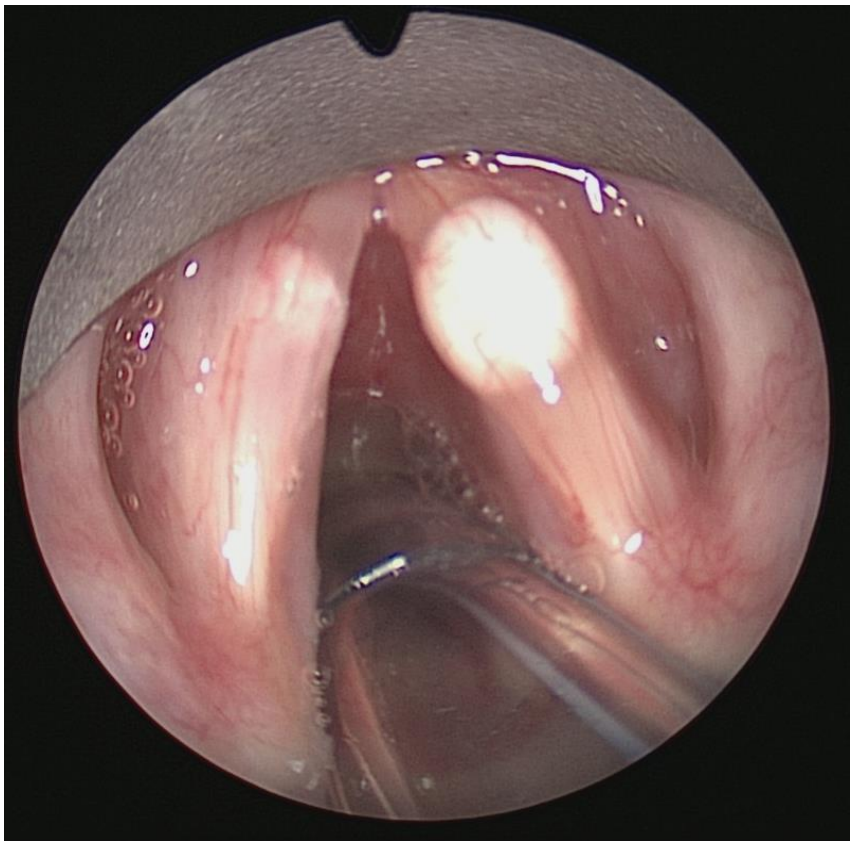


Fig. 12 Right vocal cord cyst.

VOCAL CORD PAPILLOMA

Respiratory papillomatosis is a chronic infection of viral etiology caused by Human Papilloma virus (HPV) namely HPV 6 and 11. It manifests as proliferation of benign squamous papillomas in the upper aerodigestive tract. This is more commonly seen among children. There are two forms of papilloma, an aggressive form that occurs in childhood and a less aggressive form that typically occurs in adults. They appear as cauliflower like sessile or pedunculated pinkish or whitish masses and are most commonly found in the transition zone between squamous and columnar epithelium. Ciliated epithelium undergoes squamous metaplasia when exposed to repeated trauma and this is why RRP is more common in patients with gastroesophageal reflux disease (42). Surgical removal using cold steel, microdebrider, using laser are the various treatment options available. Adjuvant treatment modalities like interferon acyclovir, ribavirin, cidofovir etc. have been reported to be effective in recurrent cases (43).

Fig. 13 Laryngeal Papillomatosis.

REINKE'S EDEMA

Reinke's oedema is a condition in which the vocal cords become chronically or irreversibly swollen. This is also termed as polypoid corditis or vocal polyposis or smokers' larynx. Reinke's oedema appears as an out pouching from the membranous vocal folds sometimes resembling a water balloon. Smoking, gastroesophageal reflux, voice strain, hypothyroidism play an important role in its development. Goswami et al (44) conducted a clinicopathological analysis of 92 cases of Reinke's oedema and reported that this was mostly found in middle aged persons with a male predilection and a strong association with smoking. Histologically Reinke's oedema is characterised by lakes of oedema in the Reinke's space with extravasated erythrocytes and thickening of the walls of the subepithelial vessels and thickening of basement membrane. A severity grading for Reinke's oedema was put forward by Savic (29).

The grading is as follows

Grade 1-Marginal edge oedema

Grade 2-Obvious sessile swelling thrown over the vocalis muscle during phonation.

Grade 3-Large bag like swellings filled with fluid.

Grade 4-Partially obstructing lesions with medial border in contact in almost entire length.

The patient presents with symptoms of change in voice with a deepening of the pitch of voice, effortful speaking and sometimes choking episodes. The voice becomes low pitched due to increase in the mass of vocal folds and the patient will be unable to produce high notes. The voice is typically said to be raspy. On stroboscopy the mucosal wave will be decreased due to the mass effect of the oedema with phase asymmetry due to ball valving and

asymmetric oedema. Treatment options include reduction glottoplasty with cessation of smoking.

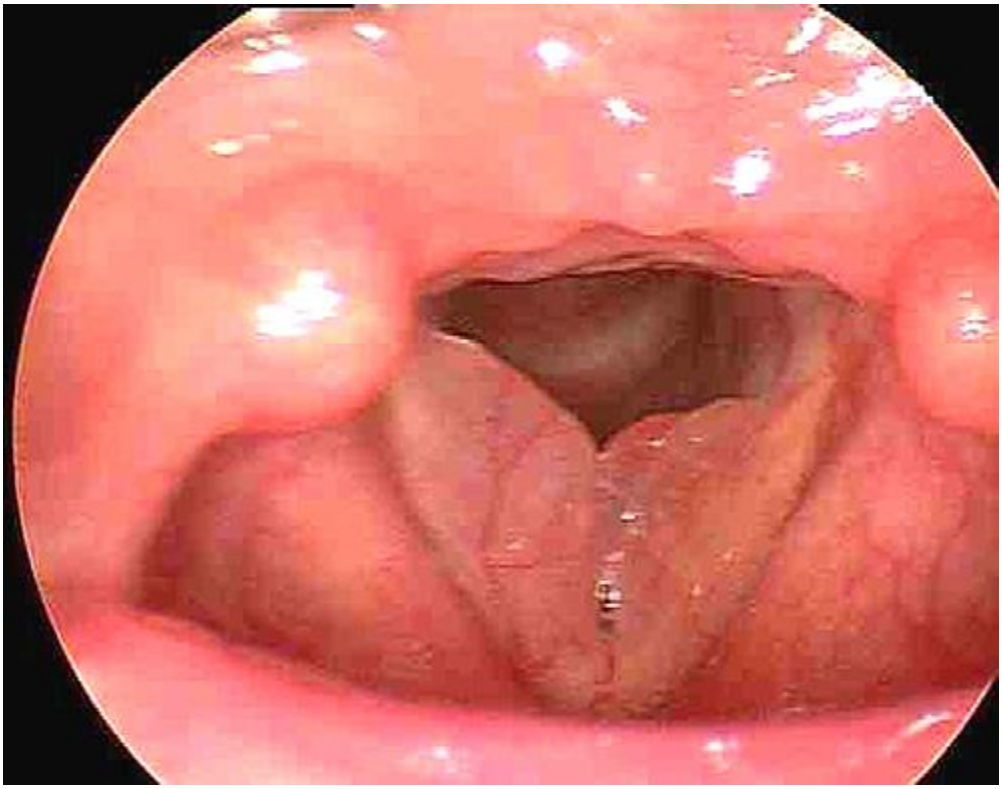


Fig. 14 Bilateral polypoid corditis (*Adopted from Otolaryngol Clin N Am40 (2007) 1091–1108)*

VOCAL CORD KERATOSIS

Vocal cord keratosis is one of the premalignant lesions of larynx. It presents as a keratinised whitish plaque on the epithelium of the vocal cord. This is also called leukoplakia of the cords. The chance of transformation into malignancy is about 8% (45). Very early lesions show hyperkeratosis and parakeratosis without cellular atypia whereas squamous dysplasia shows cellular atypia which may be mild, moderate, severe or carcinoma in situ (31). Treatment

is by microsurgical excision and biopsy. Malignant transformation of mild dysplasia is reported to be 11% where as that of severe dysplasia or carcinoma in situ is reported to be as high as 45%. So a long term follow up of such patients is absolutely necessary.

Fig. 15 Left vocal fold keratosis

GRANULOMAS

Granulomas occur following trauma to the vocal cords most commonly in patients with laryngopharyngeal reflux, with excessive throat clearing and following endotracheal intubation. They usually do not cause any voice disturbances since they are found on the cartilaginous part of the cords (38). Large granulomas can cause hoarseness and

even difficulty in breathing. Granulomas can also occur in the larynx secondary to specific granulomatous diseases of larynx like tuberculosis, leprosy, leishmaniasis, syphilis, blastomycosis etc. Intubation granulomas were more frequently found in females. This was attributed to anatomic peculiarities of female larynx such as small size allowing more contact of the endotracheal tube with the airway mucosa. Granulomas frequently occur in the posterior glottis because the perichondrium over the vocal process is very fragile predisposing to formation of ulcers (46). Histopathological examination shows mild epithelial hyperplasia with severe inflammation and vessel proliferation in the centre.

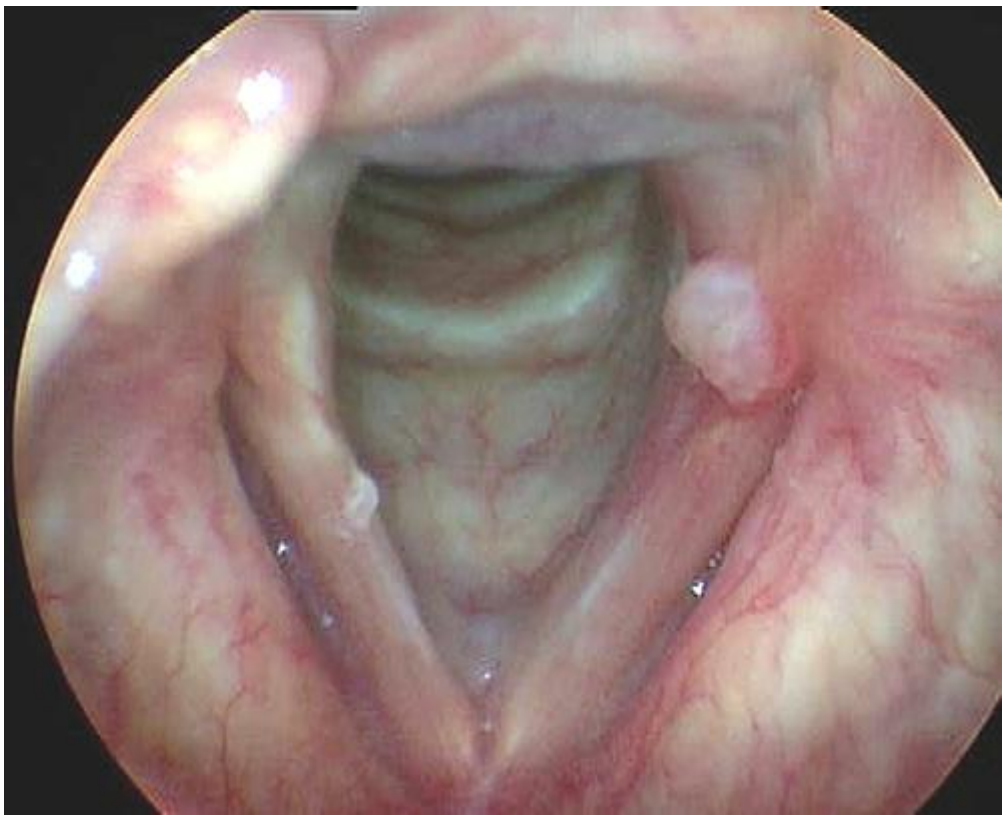


Fig. 16 Left Vocal process granuloma (*Adopted from Otolaryngol Clin N Am40 (2007) 1091–1108)*

MICROVASCULAR LESIONS

These include varices or capillary ectasias. They are usually found over the superior or medial surface of mid membranous portion of the vocal folds. They are aberrant vessels of microcirculation lying within the superficial layer of lamina propria (38). They may also be associated with vocal cord haemorrhage, polyp or even scarring. Pulsed angiolytic lasers like KTP lasers have been reported to be useful in treating such lesions.

PREMALIGNANT LESIONS OF LARYNX.

Premalignant lesions of larynx is defined by WHO as " morphological alterations of mucosa caused by chronic local irritative factors or referable to local expression of generalised illness, presenting a higher probability of degeneration into carcinoma with respect to surrounding mucosa" (47). Laryngeal precancerous lesions have no specific macroscopic appearance and are variously referred as chronic laryngitis, keratosis, leukoplakia, erythroplakia, hyperplastic-dysplastic lesions. Whatever the macroscopic appearance, the histopathological feature is the key for management of such lesions.

World Health Organisation has classified premalignant lesions of larynx into six groups.

1) Hyperplasia.
2) Keratosis.
3) Mild dysplasia.
4) Moderate dysplasia.
5) Severe dysplasia.
6) Carcinoma in situ (CIS).

Another well known classification is the Ljubljana classification. According to this the premalignant lesions are classified as

1) Simple hyperplasia.
2) Basal / parabasal hyperplasia.
3) Atypical hyperplasia.
4) Carcinoma in situ (48).

Histologically very early lesions may manifest hyperkeratosis or parakeratosis only without cellular atypia whereas dysplastic lesions demonstrate cellular atypia with loss of maturation or stratification. In mild dysplasia the changes are limited to basal one third of the epithelium whereas in moderate it involves up to two thirds of the epithelium and in severe dysplasia changes are seen in more than two thirds of the thickness of the epithelium. Carcinoma in situ is characterised by involvement of full thickness of the epithelium but without violation of basement membrane and stromal invasion.

Visual appearance of the lesion does not predict the histological nature and therefore biopsy is a must for the diagnosis and further management. The rate of malignant transformation for severe dysplasia and carcinoma in situ is as high as 30 to 45% (31). Treatment modalities include microlaryngoscopy and excision by conventional method or with CO2 laser and by radiotherapy. All these patients require a close follow up due to the high risk of malignant transformation (48).

MALIGNANT TUMOURS OF LARYNX

Laryngeal cancer is reported to be the eleventh most common cancer worldwide and second most common malignancy of upper aerodigestive tract (38). Among them glottic cancer are the most common. The incidence is about 30-35% in the supraglottic region, 60-65% in the glottis and 5% in the subglottic region. World health Organisation has put forward a histological classification for tumours of larynx. These are broadly classified into epithelial tumours, malignant salivary gland tumours, malignant soft tissue tumours, malignant tumours of bone and cartilage, haematolymphoid tumours and secondary laryngeal malignancies (31). Squamous cell carcinoma accounts for 85 to 95% of malignant laryngeal tumours arising from the epithelial lining of larynx. Glottic carcinomas are reported to be the most common in United States accounting to about 59% and subglottic carcinomas were rare accounting for about 1% (38). Laryngeal cancer is common in males with an incidence of 5.3 per 100000 population compared to females where the incidence rate is 1 per 100000 population (49).

Aetiology of Laryngeal cancer.

Smoking and alcohol are considered to be primary risk factors for laryngeal cancer. Human Papilloma virus, chemical carcinogens, prior exposure to radiation, family history of head and neck malignancies and diet are considered to be other risk factors. Smokers have a 10 to 20 fold increase in laryngeal cancer compared to non smokers and the risk sharply decreases with cessation of smoking (38). Tobacco and alcohol are the two strong etiological factors for the development of Head and Neck Squamous Cell Carcinoma acting both independently and synergistically (50). Alcohol consumption is strongly linked to supraglottic carcinoma whereas smoking is strongly associated with glottic carcinoma. The risk

is proportional to the amount and duration of tobacco or alcohol consumption and decreases slowly after cessation but does not return to baseline value for at least 20 years. It has been demonstrated that derivatives of burning cigarette such as nitrosamines and polycyclic aromatic hydrocarbons are potential carcinogens for laryngeal epithelium. They produce mutations in the DNA, disrupt the normal cell cycle leading to proliferation of tumour cells leading to carcinogenesis. Also smoking black (air cured) tobacco is associated with greater risk due to greater amount of carcinogens in it. Alcohol causes chronic inflammation of the lining of the larynx resulting in genetic mutations leading to carcinogenesis. Also chronic alcoholism leads to malnutrition, changes in immunoglobulin level and vitamin deficiencies especially Vit A and E which are important antioxidants (51).

Gastroesophageal reflux is also being considered as a risk factor for laryngeal carcinoma as this causes chronic laryngeal irritation but the causality of the association is yet to be proven. The role of reflux was more clearly demonstrated in patients with laryngeal cancer who were non smokers. A significant association with GERD was proposed in a retrospective study conducted by Freije et al (52) on patients with laryngeal cancer without any other risk factors. In a study conducted by Francis et al (53), no association was found between laryngopharyngeal reflux and laryngeal cancer. Johnston et al (54) have reported that refluxed pepsin can promote proliferation of laryngeal and pharyngeal epithelial cells .

Human Papilloma Virus (HPV) subtypes 16 and 18 have been closely related to laryngeal cancer and is considered as an independent risk factor for laryngeal cancer. HPV 16 is the predominant genotype identified in head and neck tumours. It has been associated with verrucous cell carcinoma and squamous cell carcinoma of larynx. HPV integrates into the host cell genome and the viral particles replicate in the basal cell layer. An epithelial cell infected by HPV produces more tumorigenic cells.

Occupational exposure to carcinogens in work place has been attributed to carcinoma larynx. Cement dust, fertilizers, polycyclic hydrocarbons, petroleum derivatives, wood and metal dust are potential carcinogens. Chemical solvents acts synergistically with tobacco and alcohol and doubles the risk of laryngeal cancer (55).

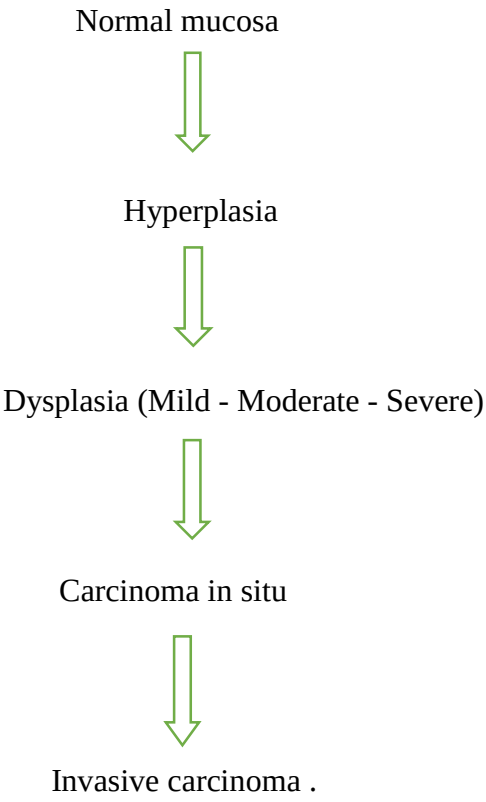
Genetic predisposition has also been shown to have a role in the pathogenesis of laryngeal cancer. A small number of patients who have no exogenous risk factors develop tumour and this can be attributed to the familial inheritance. The risk is higher when there is family history of cancer in a first degree relative and is further increased if the neoplasm was in the head and neck region (56).

Recently the role of *Helicobacter pylori* as an etiological agent for laryngeal cancer have been studied. Several studies have yielded conflicting results. *H. pylori* infection is considered to cause proliferation of epithelial cells which may progress to laryngeal carcinoma (57). Rezaii et al (58) studied the relationship between *H. pylori* seropositivity and hypopharyngeal and laryngeal carcinoma and concluded that *H. pylori* seropositivity was more in patients with laryngeal carcinoma than in the control group. Another study conducted by Genc et al (57) failed to show an association between *H. pylori* and laryngeal carcinoma. Immunohistochemical staining for *H. pylori* was done in the 59 laryngeal specimens in this study, out of which 31 were laryngeal cancer and 28 were benign laryngeal pathologies. Pirzadeh et al (1) investigated the possible role of *H. pylori* as a cause of squamous cell carcinoma of larynx by doing rapid urease test on laryngeal specimens, but failed to prove an association as an etiological agent for laryngeal cancer.

The multiple aetiological factors act on the various levels of the molecular structure of the epithelial cell lining of larynx and act as carcinogens. Thus a series of events

occur and there is a progression of normal mucosa to pre malignant lesions and then to invasive cancer.

Progression of normal epithelium to premalignant and to invasive disease in squamous cell carcinoma (59)



A variant of squamous cell carcinoma is verrucous carcinoma. It appears as a predominant exophytic growth and histologically characterised by well differentiated keratinising epithelium with pushing margins. The squamous epithelium lacks the cytological criteria for malignancy which differentiates it from conventional squamous cell carcinoma. Verrucous carcinoma usually do not metastasize to cervical lymph nodes. The treatment of choice is surgical excision as these tumours are less radiosensitive (31). Basaloid squamous cell carcinoma is a rare and highly malignant form of squamous cell carcinoma more commonly involving the supraglottic larynx and pyriform sinus. Other variants of SCC includes adenosquamous carcinoma, acantholytic squamous carcinoma and papillary squamous carcinoma.

A typical patient with laryngeal cancer presents with hoarseness, dysphagia, odynophagia, dyspnoea, referred otalgia and aspiration. Glottic carcinomas present earlier than supraglottic and subglottic tumours because any small lesion in the vocal cord can cause change in voice. Supraglottic tumours usually presents with dysphagia, odynophagia and neck nodes whereas subglottic tumours commonly presents with airway obstruction (60). Hoarseness in supraglottic and subglottic tumours will be a late manifestation.

Supraglottic tumours may even present with cervical lymphadenopathy whereas glottis tumours seldom presents with lymphadenopathy because of poor lymphatic supply to glottis. Supraglottic tumours usually metastasize to level 2, 3 and 4 lymph nodes. Laryngeal examination shows an ulcerative, exophytic or polypoidal lesion. Sometimes it can even present as a laryngocoele. Carcinoma arising from the ventriculosaccular region may present as a fullness of the ventricular region (31).

Evaluation of a patient presenting with laryngeal carcinoma begins with a thorough history and clinical examination. Flexible fibreoptic laryngoscopy is extremely

helpful as it helps to visualise the hidden areas of larynx and permits photographic and video documentation. Imaging provides additional information which helps in staging of the disease and further surgical planning. CT and MRI are the major imaging modalities which helps in visualisation of extent of disease (60). MRI is superior to CT in assessing cartilage invasion and discrimination of soft tissue structures.

Staging the disease is very important for optimal treatment. The TNM staging for laryngeal carcinoma is as follows:

Primary tumour (T)

- Tx- Primary tumour cannot be assessed.
- T0- No evidence of primary tumour.
- Tis- Carcinoma in situ.

Supraglottis:

- T1-Tumour limited to one subsite of supraglottis with normal vocal cord mobility.
- T2-Tumour invades mucosa of more than one adjacent subsite of supraglottis or glottis or region outside the supraglottis (mucosa of base of tongue, vallecula or medial wall of pyriform sinus) with normal vocal cord mobility.
- T3-Tumour limited to the larynx with vocal cord fixation and/or invades any of the following: postcricoid area, pre epiglottic tissue, paraglottic space and /or minor thyroid cartilage erosion (inner cortex)
- T4a-Tumour invades through the thyroid cartilage and/or invades tissues beyond the larynx (eg. trachea, soft tissues of the neck including deep extrinsic muscles of the tongue, strap muscles, thyroid or oesophagus.)

- T4b-Tumour invades prevertebral space, encases carotid artery, or invades mediastinal structures.

Glottis:

- T1-Tumour limited to the vocal cords, may involve anterior and posterior commissure, with normal vocal cord mobility

  T1a-Tumour limited to one vocal cord.

  T1b-Tumour involves both vocal cords.
- T2-Tumour extends to supraglottis and/or subglottis or with impaired vocal cord mobility.
- T3-Tumour limited to the larynx with vocal cord fixation and/or invades paraglottic space and/or minor thyroid cartilage erosion (inner cortex).
- T4a-Tumour invades through thyroid cartilage and/or invades tissues beyond the larynx. (eg. trachea, soft tissues of the neck including deep extrinsic muscles of the tongue, strap muscles, thyroid or oesophagus.)
- T4b-Tumour invades prevertebral space, encases carotid artery, or invades mediastinal structures.

Subglottis:

- T1-Tumour limited to the subglottis.
- T2-Tumour extends to vocal cords with normal or impaired cord mobility
- T3-Tumour limited to larynx with vocal cord fixation.

- T4-Tumour invades cricoid or thyroid cartilage and/or invades tissues beyond the larynx (eg. trachea, soft tissues of the neck including deep extrinsic muscles of the tongue, strap muscles, thyroid or oesophagus.)
- T4b-Tumour invades prevertebral space, encases carotid artery, or invades mediastinal structures.

Regional lymph nodes (N)

- Nx- Regional lymph nodes cannot be assessed.
- N0- No regional lymph node metastasis.
- N1- Metastasis in a single ipsilateral lymph node less than or equal to 3cm in greatest dimension.
- N2-Metastasis in a single ipsilateral lymph node >3cm but not >6cm in greatest dimension, or in multiple ipsilateral lymph nodes none >6cm in greatest dimension, or in bilateral or contralateral lymph nodes, none >6cm in greatest dimension.
- N2a- Metastasis in a single ipsilateral lymph node >3cm but not >6cm in greatest dimension.
- N2b-Metastasis in multiple ipsilateral lymph nodes none >6cm in greatest dimension.
- N2c- Metastasis in bilateral or contralateral lymph nodes, none >6cm in greatest dimension.
- N3-Metastasis in a lymph node >6cm in greatest dimension.

Distant metastasis (M)

- Mx-Distant metastasis cannot be assessed.
- M0-No distant metastasis
- M1-Distant metastasis

Treatment of Laryngeal Cancer

A microlaryngoscopy and biopsy gives a histopathological diagnosis followed by which definitive treatment is planned. Treatment modalities include surgery, radiation, chemotherapy or a combination of the three. Management depends upon the stage of the tumour. As a general rule, early disease is managed by single modality treatment whereas advanced disease is managed by combined modality treatment. Larynx has got indispensable role in speech and communication and so preservation of voice is very important in management of early laryngeal cancer. Finally the choice of treatment modality depends on patient's wishes, functional outcome, reliability in follow up and patient's general condition.

According to AJCC, early stage glottis tumours are T1 and T2 tumours with no regional lymph node involvement and lacking distant metastasis. Early glottis tumours can be treated surgically or by radiotherapy. Surgical options have evolved from partial laryngectomies by external approaches in the past to transoral endoscopic laser resections. Transoral laser resection has established its role in the management of early laryngeal cancer. With carbon dioxide laser under microscopic magnification the lesion is removed in piecemeal. With laser excision, the postoperative pain is very minimal. Other advantages include short treatment time and short duration of hospital stay.

The European Laryngological Society (ELS) Working Committee has classified cordectomies into following categories (61).

Type I: Subepithelial cordectomy (resection of the epithelium alone)

Type II: Subligamental cordectomy (resection of epithelium, Reinke's space and vocal ligament)

Type III: Transmuscular cordectomy (resection through the vocalis muscle)

Type IV:Total cordectomy (resection of the cord up to the anterior commissure)

Type V: Extended cordectomy. This is sub classified as

Va: resection up to the contralateral vocal cord including the anterior commissure.

Vb: resection including arytenoids.

Vc: resection including subglottis.

Vd: resection including the ventricle.

Type VI: Anterior commissurectomy with bilateral anterior cordectomy.

Also patients can be offered revision laser surgeries, conservational laryngeal surgeries, total laryngectomy or radiotherapy in case of persistent or recurrent disease. Open surgical procedures for early glottic cancer are classified as organ preservation surgeries with the aim of preserving speech and swallowing and avoiding creation of a permanent tracheostomy. The prerequisite for all patients who are planned for conservational laryngeal surgeries is an adequate pulmonary reserve as all of them will face varying degrees of aspiration during the postoperative period. The surgical procedures include cordectomy, vertical partial hemilaryngectomy, frontolateral hemilaryngectomy and supracricoid laryngectomy.

The most important prognostic factor is the stage of the disease and the N stage is more important in predicting the survival than the T stage. Other important prognostic factor is the site of the disease. Glottic carcinoma has got the best prognosis whereas subglottic cancers have the worst prognosis (31). All laryngeal cancer patients require a strict follow up. Most of the recurrences occur in the first 2 years after primary treatment. According to NCCN guidelines, the follow up for laryngeal cancer is every 1-3 months for the first year, then every 6 months for the next one year and yearly for the next 3 years.

Fig. 17. Carcinoma of the left vocal cord.

GASTROESOPHAGEAL REFLUX DISEASE.

Gastroesophageal reflux is defined as the retrograde movement of gastric contents into the oesophagus in the absence of belching or vomiting. When the reflux is manifested with clinical symptoms and complications, it is termed gastroesophageal reflux disease. When the reflux affects the laryngopharynx, it is termed as laryngopharyngeal reflux

(62). GERD is a multifactorial disease where dietary factors, environmental factors and host factors play a very important role. A thorough knowledge of the anatomy and physiology of oesophagus is essential to understand the pathology of reflux disease.

Anatomy of oesophagus.

The oesophagus is a muscular tube connecting the pharynx to the stomach and acts as a conduit for the passage of material from pharynx to the stomach. Upper oesophageal sphincter (UES) forms the proximal margin of tubular oesophagus correlating anatomically with the junction of inferior constrictor with cricopharyngeus. The oesophagus extends distally 18-26 cm from the UES through the posterior mediastinum to end in the lower oesophageal sphincter. Upper oesophageal sphincter is normally closed and opens for specific physiologic demands as swallowing or belching. UES is the final gatekeeper of antireflux mechanism. Once it fails functioning all the head and neck manifestations of reflux disease appear. The lower oesophageal sphincter (LES) is not anatomically distinct as the upper oesophageal sphincter and is a 2-4 cm long focus of tonically contracted thickened circular smooth muscle lying within the diaphragmatic hiatus. LES is the most critical antireflux defence mechanism protecting oesophagus from reflux of gastric contents. Contraction of LES results in circular closure whereas relaxation occurs during swallowing, belching or vomiting. LES should maintain a higher resting pressure than stomach to prevent reflux. GER occurs when there is a reversal of gastric to LES pressure gradient (63).

Histologically oesophagus is composed of four layers namely mucosa, submucosa, muscularis propria and adventitia. The oesophagus lacks serosa which makes it unique to the rest of gastrointestinal tract. The muscularis propria is composed of both skeletal muscles and smooth muscles. The proximal 5% to 33% is skeletal muscle, the middle 35% to 40% is mixed and the distal 50% to 60% is smooth muscle. These muscles are arranged into

inner circular and outer longitudinal layer. Vagus nerve innervates the smooth muscle and thereby controls the peristalsis under physiologic conditions. Oesophagus is also innervated by two nerve plexus namely Meissner's plexus and Myenteric plexus. Meissner's plexus is located in the submucosa and the Myenteric plexus or Auerbach's plexus is located between two muscle layers (64). The Meissner's complex is the site of afferent sensory input whereas myenteric plexus is concerned with oesophageal peristalsis. Excitatory stimulation from acetylcholine results in contraction of circular and longitudinal muscle fibres, whereas inhibitory neurons mainly affect the circular muscle fibres via nitric oxide.

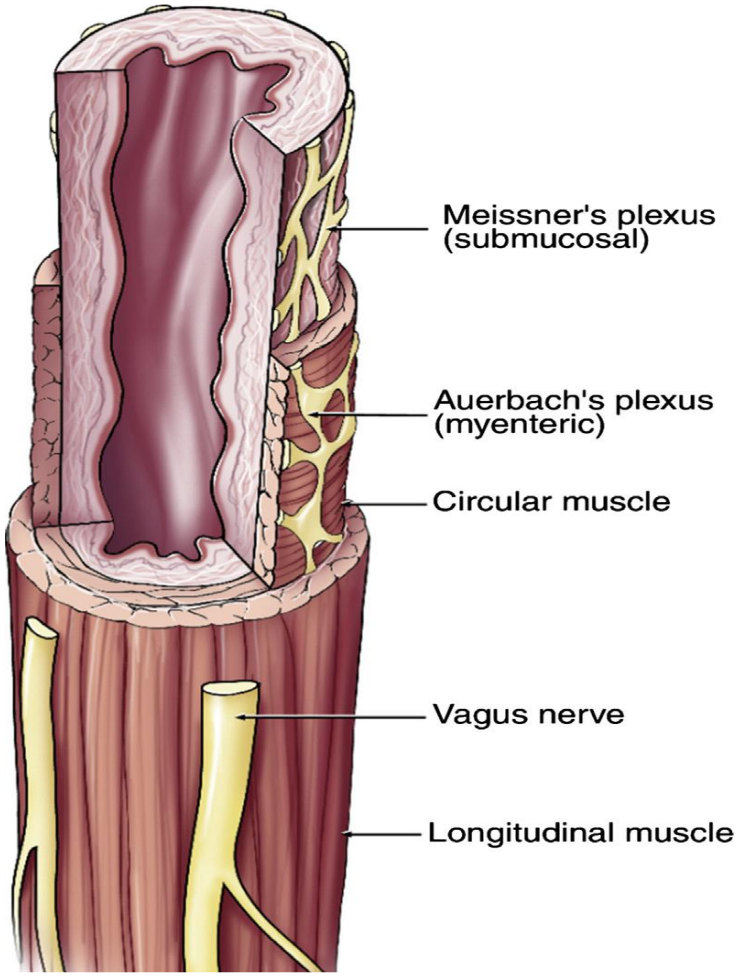


Fig. 18. Cross sectional anatomy of oesophagus (*Adopted from Otolaryngol Clin North Am. 2013 Dec; 46(6):1023–41. Normal oesophageal physiology and laryngopharyngeal reflux.)*

OESOPHAGEAL PHYSIOLOGY

Functionally, the UES, the oesophageal body, and the LES acts in a coordinated manner to allow normal swallowing. Swallowing begins when a food bolus is propelled into the pharynx from the mouth. The oropharyngeal phase of swallowing is voluntary, whereas the oesophageal phase is involuntary. During swallowing, the larynx is elevated and the epiglottis seals the airway. A rapidly progressing pharyngeal contraction then transfers the bolus through the relaxed UES into the oesophagus. As the UES closes, a progressive circular contraction begins in the upper oesophagus and proceeds distally along the oesophageal body to propel the bolus through the relaxed LES. Peristaltic pressures normally ranging from 30 to 180 mmHg are generated (65).The measured pressure tends to be lower in the more proximal portions of the oesophagus and greater in the distal smooth muscle portions. The pressures may also vary with the consistency of the bolus itself. The LES subsequently closes with a prolonged contraction, preventing movement back into the oesophagus. The mechanical effect of peristalsis is a stripping wave that strips the oesophagus clean from its proximal to its distal end. Secondary peristalsis is a progressive contraction in the oesophageal body that is induced by stimulation of sensory receptors, rather than a swallow. Distention by residual food bolus or the refluxed gastric contents are usually the stimulants.

Pathophysiology of GERD.

Gastroesophageal reflux disease is a very common disease entity nowadays and approximately 25 million to 75 million people in the United States are affected by this disease. GERD usually presents with a wide variety of symptoms, the most common being heartburn

and regurgitation. Several factors are being implicated in the pathogenesis of GERD which include oesophagogastric junction, oesophageal acid clearance, oesophageal mucosal resistance, upper oesophageal sphincter. As explained earlier, the lower oesophageal sphincter is responsible for maintaining a high pressure zone between the oesophagus and the stomach. The LES relaxes normally during swallowing and allows the passage of food bolus into the stomach. Sometimes the LES relaxes independent of swallowing. This is termed as transient LES relaxation and this lasts longer than normal LES relaxation. They occur in response to vagal sensory and motor nerve stimulation due to gastric distension (66). This transient relaxation is considered to be most important pathology in patients with GERD. In them, the transient relaxations occur more frequently than normal.

The crural diaphragm provides structural support and contributes to LES competence. The presence of a hiatal hernia widens the diaphragmatic hiatus and impair the function of crural diaphragm as an external sphincter worsening the reflux. LES pressure can also be altered with fatty foods, ethanol, cola, drugs like calcium channel blockers, diazepam etc. Thus a defective lower oesophageal sphincter increases the volume of gastric contents that reflux into the oesophagus.

Increased oesophageal acid exposure is another factor considered in the pathophysiology of GERD. Oesophageal acid clearance occurs normally by means of oesophageal peristalsis and swallowed salivary bicarbonate. Peristalsis clears the gastric refluxate and salivary bicarbonate neutralises the remaining acid (66). Prompt clearance of the refluxate reduces the duration of the oesophageal mucosa being exposed to the acid. Both primary and secondary oesophageal peristalsis contributes to oesophageal acid clearance.

Peristaltic dysfunction and re- reflux are thought to be two mechanisms of impaired acid clearance. It has been shown that the frequency of secondary oesophageal peristalsis was lower in patients with GERD.

Another factor that protects the oesophageal mucosa from the refluxate is the resistance offered by the oesophageal mucosa. This resistance is offered by a complex of layers formed by the mucus, unstirred water layer, cell membrane, intercellular bridges at the epithelial level, and the buffering system all of which acts as a barrier to mucosal injury. The oesophagus also produces bicarbonate which buffers the acid. Any breach in this barrier can aggravate the reflux manifestations (62). Pepsin is said to damage the oesophageal mucosa by digesting epithelial protein.

GERD has been associated with multiple risk factors. Studies have reported that male sex has a higher incidence of oesophagitis. Obese individuals and those with hiatus hernia are more prone for reflux symptoms. The presence of a hiatal hernia makes the lower oesophageal sphincter less competent with defective peristalsis leading to acid reflux and increased mucosal damage. Also an association with scleroderma and patients with chronic obstructive pulmonary disease is being described. Pregnancy is another risk factor for GERD.

GERD is broadly classified into two categories based on the findings on endoscopy. They are those with oesophageal mucosal damage which include Barrett's oesophagus and erosive esophagitis and those without oesophageal mucosal damage. This is also known as Endoscopy negative reflux disease or Non erosive reflux disease or NERD. In

other words, GERD can be considered as a spectrum of disease where NERD lies at the mild end of the spectrum and erosive esophagitis lies at the other end (67).

The Society of American Gastrointestinal Endoscopic Surgeons (SAGES) have put forward the following guidelines for the diagnosis of GERD on endoscopy. These include presence of mucosal injury on endoscopy in patients with typical symptoms, Barrett's oesophagus on biopsy, peptic stricture after excluding malignancy or a positive pH metry. But this criteria fails to identify patients with NERD. The severity of mucosal injury on endoscopy is graded into four categories according to the Los Angeles classification (19).

Grade 1- Presence of one or more mucosal breaks, none longer than 5 mm and none of which extends between the tops of mucosal folds.
Grade 2- Presence of one or more mucosal breaks longer than 5 mm but none of them extends between the tops of mucosal folds.
Grade 3- Presence of mucosal breaks that extends between the tops of two or more mucosal folds but involves less than 75 % of the oesophageal circumference.
Grade 4- Mucosal breaks involve at least 75% of the circumference of oesophagus.

Laryngopharyngeal reflux (LPR) is the extraesophageal variant of GERD and patients presents with symptoms that are different from symptoms of GERD. Most of the patients even deny ever having a heartburn. The usual symptoms in LPR include change in voice, globus sensation, chronic throat clearing, sore throat, excessive throat mucus, chronic cough, neck pain, voice fatigue and voice breaks etc. (31). Larynx is highly vulnerable to reflux as it lacks protective mechanisms of oesophagus such as intrinsic acid clearing mechanism, production of bicarbonate and epithelial resistance barrier (63). Reflux is believed to damage

the larynx directly and indirectly. Direct contact by acid and pepsin with laryngeal mucosa results in laryngeal mucosal injury(microaspiration theory) (68). On the other hand, irritation of the distal oesophagus triggers a vagal response that results in chronic cough (oesophageal bronchial reflex theory) and recurrent throat clearing that indirectly traumatises the laryngeal mucosa (69).

LPR has been associated with a numerous laryngeal pathologies like muscle tension dysphonia, Reinke's oedema, globus pharyngeus, posterior laryngitis, diffuse laryngitis, subglottic stenosis, cricoarytenoid joint ankylosis, carcinoma etc. Chung et al (70) studied the significance of laryngopharyngeal reflux in patients with benign vocal cord lesions and concluded that reflux might play a role as an etiological factor for Reinke's oedema and vocal cord polyps. They studied the presence of reflux in patients with vocal cord lesions like Reinke's oedema (n=20), vocal cord polyps (n=40) and vocal cord nodules (n=50) and compared to a control group of LPR symptomatic patients with no signs of laryngeal lesions on fibreoptic laryngoscopy. They concluded that the prevalence of reflux on dual probe pH monitoring was more in people with laryngeal lesions. Beltsis et al (71) conducted a double probe pH monitoring in patients with benign vocal cord pathologies and concluded that acid reflux was more prevalent in patients with these pathologies.

Although dual probe pH monitoring is considered to be the gold standard for diagnosing extraesophageal reflux, it has got its own limitations. It is an invasive test as it requires placement of electrodes endoscopically. Also its sensitivity is not more than 75 to 80%. Koufmann and colleagues have developed a self-administered nine item Reflux Symptom Index questionnaire to assess the severity of reflux and to compare the results after treatment (72). The assessment can be completed in less than a minute. The scale for individual item varies from 0 (no problem) to 5 (severe problem) with a maximum total score of 45.

The questions included are

1. Hoarseness or problem with your voice.
2. Clearing your throat.
3. Excess throat mucus or postnasal drip
4. Difficulty swallowing foods, liquids or pills.
5. Coughing after you ate or after lying down.
6. Breathing difficulties or choking episodes.
7. Troublesome or annoying cough.
8. Sensations of something sticking in throat or lump in throat.
9. Heartburn, chest pain, indigestion or stomach acid coming up.

The Reflux Finding Score is an eight item score developed by Belafsky to standardise the laryngeal findings in LPR to assess the clinical severity and clinical improvement and therapeutic efficacy after appropriate treatment. These 8 items were selected from a pool of most common laryngeal findings seen in LPR patients (73).The scoring is done by the surgeon after a laryngeal examination.

The scoring is as follows

1.Subglottic oedema	0-absent 2-present
2.Ventricular obliteration	2-partial 4-complete
3.Vocal fold oedema	1-mild 2-moderate, 3-severe 4- polypoidal
4. Erythema/Hyperaemia	2-arytenoids only 4-diffuse.
5.Posterior commissure hypertrophy	1-mild 2-moderate 3-severe 4-obstruction.
6. Diffuse Laryngeal oedema	1-mild 2-moderate 3-severe 4-obstruction
7. Granuloma/granulation	0-absent 2-present.
8.Thick endolaryngeal mucus	0-absent 2-present.

Total Score : 0-26.

A score of 7 indicated possible presence of laryngopharyngeal reflux.

Koufmann in 1995 first described subglottic oedema. It is also called pseudo sulcus vocalis. Vocal cord with pseudo sulcus is classically described as having the appearance of a partially open hot dog bun. The oedema is not really subglottic. Subglottic oedema extends from anterior commissure to posterior larynx. True sulcus or sulcus vergeture extends only up to the vocal process and is more in the midzone or striking zone of vocal cords whereas pseudo sulcus extends all the way posteriorly and is inferior to the striking zone. Presence of pseudo sulcus contributes 2 points to the RFS. Pseudo sulcus is also seen in aging larynx. Pseudo sulcus as the only finding is very rare in LPR.

Ventricular obliteration is another common finding in patients presenting with LPR. Ventricle is the space between true and false vocal cords. Swelling of the true cords and the false cords causes obliteration of the ventricular space. It is said to be partial when the ventricular space is reduced and the false cord edge is indistinct and complete when the true and false cords appear to touch each other and there is no true ventricular space. Partial obliteration is scored as 2 and complete obliteration is scored as 4.With effective antireflux treatment this finding reverses and the ventricular bands become sharp and angular

Erythema or hyperaemia of larynx or posterior laryngitis is the classic finding described in patients presenting with reflux. Isolated erythema of arytenoids is scored as 2 whereas diffuse erythema is scored as 4. But this is a relatively nonspecific finding as it is significantly dependent on the video endoscopic equipment used. Vocal fold oedema is graded as mild [1] if only slight swelling is present, moderate [2] if the swelling is more perceptible, severe [3] when the swelling of the cord becomes sessile. Polypoidal degeneration of vocal cord contributes 4 points to RFS.

Diffuse laryngeal oedema is assessed by the size of the airway relative to the size of the larynx and is graded as mild, moderate, severe and obstructing. Posterior commissure hypertrophy is another frequent finding in LPR. It is graded as mild when there is moustache like appearance of posterior commissure mucosa, moderate when the mucosa is swollen enough appearing like a straight line across the back of larynx, severe when there is bulging of posterior larynx into the airway and obstructing when a significant portion of airway is obliterated. Granuloma or granulation tissue and thick white endolaryngeal mucus anywhere in the larynx is graded as positive reflux finding.

In a study conducted by Cekin et al (74), 43 patients with laryngeal pathologies were selected and they were evaluated for gastroesophageal reflux using the Reflux symptom questionnaire. They underwent microlaryngoscopy and biopsy for definitive diagnosis of the laryngeal pathology and the biopsy tissue was also tested for presence of *H. pylori* by PCR analysis. Reflux was found to be present in 30 out of the 43 patients and *H.pylori* was present in 18 out of 30 reflux positive patients and 6 of the 13 reflux negative patients. There was no statistically significant difference between reflux positivity and *H. pylori* status according to this study.

Diagnosis of LPR

Ambulatory pH monitoring:

Ambulatory 24 hour double probe pH monitoring is the investigation of choice for the diagnosis of laryngopharyngeal reflux. In this, the distal probe is placed 5cm above the lower oesophageal sphincter (LES) and the proximal probe in the hypopharynx about 1cm

above the upper oesophageal sphincter just behind the laryngeal inlet. The traditional technique was to place the probe under manometric guidance. In this, a manometer is passed through the nasal cavity in to the stomach. It is then slowly withdrawn and the locations of LES and UES are determined and the correct pH catheters are then placed based on the measurements.

In the modified technique, the proximal probe is placed above the upper oesophageal sphincter under fibreoptic guidance. The distal probe is fixed 15cm below the proximal probe. The percentage of time the pH is less than 4 is estimated .The measurement is done for the time in upright position, supine position and the total time. The upper limit of normal for the upright period is 8% and that for supine period is considered to be 2.5%. Any reflux detected by the proximal probe is considered to be LPR (75).

Barium oesophagram:

This is an inexpensive and non-invasive test to diagnose the structural and functional abnormalities of the oesophagus. It is useful in the evaluation of oesophageal motility and clearance, presence of reflux and changes related to it. The sensitivity of barium oesophagram to detect reflux is between 20% and 60% with a specificity of 64% to 90% (31).

Oesophageal Endoscopy:

Oesophageal endoscopy provides visualisation of oesophageal mucosa and presence of oesophagitis, hiatal hernia, Barrett's oesophagus etc. Endoscopy fails to identify patients with NERD. Ambulatory pH monitoring can pick up these candidates.

Radionucleide scintigraphy:

This detects the fraction of administered radioactive material refluxed into the oesophagus with the help of a gamma camera. This test was less sensitive compared to pH monitoring and barium study and hence is not routinely used (75).

Treatment of LPR.

The goals of treatment are symptomatic relief, healing of esophagitis, prevention of complications and recurrence.

There are 3 levels of treatment for LPR.

Level I - dietary and lifestyle modification along with antacids.

Level II - Level I with use of a H2 receptor antagonist.

Level III- Antireflux surgery or proton pump inhibitor therapy.

Treatment for LPR should be individualised depending on the severity of patient's symptoms. LPR patients should be advised to avoid coffee, tea, fried and spicy foods, alcohol etc. Patient should be advised against lying down within 2 hours of the last meal. Lifestyle modifications include avoidance of over eating, elevation of head end of the bed, quitting tobacco usage, weight reduction etc. If initial treatment with Level I and II fails, Level III treatment should be initiated. This include acid suppressive therapy (75). Proton pump inhibitors are now considered to be the most effective antireflux medications, because unlike H2 receptor antagonists, they produce total acid suppression. They bind to the H+ K+ ATP ase

and inhibits the final step in acid production. The drugs commonly used are omeprazole, rabeprazole, esomeprazole, lansoprazole and pantoprazole. They have a potent and constant effect on gastric acid secretion with minimum side effects. The recommended treatment for LPR is a twice daily dosing of PPI for a period of 3 to 4 months. This can be even given up to 6 months to 1 year. Symptoms usually improve before the laryngeal findings subside. In those patients with delayed gastric emptying, prokinetic agents like metoclopramide and domperidone can be added, but they should not be used on a long term basis because of the adverse effects (67).

If there is no improvement with medications, antireflux surgery should be considered. PPI effectively reduces acid reflux, but the larynx and pharynx is still exposed to pepsin and bile reflux. In such cases antireflux surgery can be considered. The main procedure is Nissen's fundoplication which can be open or laparoscopic. In this the fundus of stomach is wrapped around the lower oesophageal sphincter which acts as the antireflux barrier (20).

MATERIAL AND METHODS

This prospective observational study was conducted in the Department of Otorhinolaryngology, Christian Medical College, Vellore which is a tertiary referral centre in Tamil Nadu. Approval of the Institutional Review Board at Christian Medical College was obtained in October 2013 (IRB Min No. 8449). Patients with laryngeal pathologies presenting to the outpatient department were briefed about the research project and requested to participate in the study.

The study was commenced after the real time PCR kit to detect the presence of *Helicobacter pylori* was procured by the Microbiology Department.

STUDY DESIGN

Observational study.

STUDY PERIOD

December 2013 to August 2014.

SETTING

The study was conducted at the Department of ENT, Departments of Microbiology and Pathology, Christian Medical College and Hospital, Vellore.

PARTICIPANTS

All patients who presented to the ENT outpatient department with complaints of change in voice and who were scheduled for microlaryngoscopic surgery were enrolled in the study after obtaining an informed consent.

Inclusion criteria:

- Patients diagnosed with laryngeal pathologies - benign or malignant ,
- Scheduled to undergo Microlaryngoscopy and biopsy/ excision biopsy.
- Age above 18 years.

Exclusion criteria:

- Patients on treatment with proton pump inhibitors or H2 antagonists or antibiotics within a period of 4 weeks.
- Patients with recent history of gastrointestinal bleeding
- Patients who have received radiation therapy.

SAMPLE SIZE CALCULATION

From literature review done, the average prevalence of *Helicobacter pylori* infection in laryngeal pathologies was about 40%

By applying the formula,

$$\mathbf{n= Z^2PQ/ d^2,}$$

where n=sample size

Z=95% confidence limits,

P=prevalence, Q=100-P, d=precision (10%),

The required sample size was calculated to be 100

STATISTICAL ANALYSIS

The qualitative variables were expressed using frequencies and percentages. The prevalence of *Helicobacter pylori* in laryngeal pathologies was calculated using frequencies and percentages with 95% confidence limits. Chi-square test was done on Reflux symptom index questionnaire with Reflux Finding Score to determine any association between the two.

METHODOLOGY

Patients attending the ENT outpatient clinic with complaints of change in voice fulfilling the criteria for inclusion were recruited into the study after obtaining an informed valid consent. A detailed history was obtained from the subjects and clinical evaluation of the patient was done. Information regarding the age, sex, occupation, level of voice use, history of belching, voice abuse, drug history and substance addictions was documented. Patients were categorised in to one of the following level of voice users.

Level of voice use:

Level I - elite vocal performers (singers, actors)

Level II - professional voice users (teachers, lecturers, barristers)

Level III - non vocal professionals (businessman, doctors, lawyers)

Level IV - non vocal non professionals (housewives and farmers)

The baseline data collected was entered in the clinical research form.

Reflux Symptom Index (RSI) questionnaire was used to assess the presence of reflux symptoms. The symptoms were scored on a self - rated RSI questionnaire on a 6 point scale from 0 = no problem to 5 = severe problem. The symptoms included hoarseness, throat clearing, excess throat mucus or post nasal drip, swallowing difficulty, cough after eating, breathing difficulty, annoying cough, foreign body sensation throat, heartburn or chest pain.

Koufman Reflux Symptom Index of more than 13 indicated the presence of laryngopharyngeal reflux.

Presence of laryngopharyngeal reflux in these patients was assessed by the Reflux Finding Score (RFS) by flexible laryngoscopy (Karl Storz, Germany).The scoring was done by the doctors posted in the ENT Endoscopy room.

The recruited subjects were then scheduled for a microlaryngoscopy and biopsy of the laryngeal lesion under general anaesthesia.

MICROLARYNGOSCOPY

Patient was intubated with an appropriate size endotracheal tube to maintain anaesthesia as well as assist the surgeon with adequate operating space. Patient was positioned supine on the operating table in such a way that the cervical spine is flexed and atlanto-occipital joint is extended. The eyes were carefully taped to prevent corneal abrasions and other injuries. The head was draped with sterile drapes. A tooth guard was introduced to protect the teeth and alveolus from the pressure of laryngoscope. The direct laryngoscope was then held in the left hand of the surgeon and the scope passed gently through the right

side of the mouth, pushing the tongue to the left and thus visualising the uvula. Once uvula was visualised, the laryngoscope was pressed into the tongue base which brings the tip of epiglottis into view. The epiglottis was lifted up with the scope and the scope advanced into the endolarynx following the endotracheal tube. This brought the arytenoids and false vocal folds into view. The scope is advanced further till it reached just above the level of vocal cords. With the aid of the chest suspension the laryngoscope is fixed and fine adjustments were made so that the lesion was well visualised.

Under microscopic visualisation, biopsy was taken from the lesion and sent for histopathological examination. A small tissue was separately taken in eppendorf microtubes for PCR analysis for *H. pylori*. The tubes were carried to the microbiology lab in special ice containers.

Fig. 19 Microlaryngoscopy setting in the operating room.

DNA extraction

Biopsy samples were stored at -70°C until the DNA extraction was done. DNA was extracted from the biopsy homogenate using the QIAamp DNA minikit by using the blood and body fluid protocol (Qiagen, Hilden, Germany)

Real-Time PCR

DNA extracts from the biopsy samples were subjected to real-time PCR, which was performed in a step-one real time PCR (Applied bio system) using the genesig kit for *Helicobacter pylori*. The kit is provided with 2x qPCR master mix and primer/probe mix specific for the detection urease encoding *ureA* gene subunit in the bacteria. The primer and probe mix works on the basis of TaqMan principle. During PCR amplification, forward and reverse primers hybridize to the DNA/cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, cleavage of probe occurs which results in the separation of reporter and quencher dye. As a result, increase in fluorescence can be detected on a range of real-time PCR platforms through the FAM channel. The kit is also provided with positive control to ensure that the primers and probes for detecting the target *UreA* gene in *H. pylori* are working properly.

For *UreA* detection, the 20 µl reaction mixture was prepared as follows: 10 µl of qPCR master mix, 1 µl of primer/probe mix, 4 µl of RNAse/DNAse free water and 5 µl of DNA extract with the concentration of 5ng/µl.

The amplification reaction was performed with the enzyme activation at 95°C for 15 mins, followed by 50 cycles consisting of denaturation at 95°C for 10 sec and annealing at 60°C for 1 minute. Samples were run in duplicates and were considered positive if at least one of the reactions was positive.

Fig. 20 PCR machine

Result interpretation

Real-time PCR for detection urease protein encoding *UreA* gene in *Helicobacter pylori* was done with genesig kit. Presence or absence of the target is the endpoint experiments in which fluorescence data are collected after the PCR is complete. During the PCR, the fluorogenic probes anneal specifically to the complementary target between the forward and reverse primer sites on the template DNA. During extension, AmpliTaq Gold DNA polymerase cleaves the hybridized probes in each sample containing the target. The cleavage of each matched probe separates the reporter dye from the quencher dye, resulting in increased fluorescence by the reporter. After PCR cycling, the StepOne™ and StepOnePlus™ instruments read the fluorescence generated during the PCR amplification. The fluorescent signals are used to determine the presence or absence of the target in each sample. The sample is considered to be positive for the target showing CT value of 26 ± 3 with corresponding sigmoid curve. Absence of target in the sample will be indicated as showing either no amplification curve or curve with lower CT value.

Modified Giemsa staining

An additional modified Giemsa staining was done on the sample sent for histopathological examination. 2- 4 μm thickness paraffin sections were prepared and stained by modified Giemsa technique. Giemsa stain was prepared by mixing 1 gram of Giemsa powder with 66 ml of glycerine. The mixture was then incubated at 60^0C for 2 hours. Once the temperature of the mixture drops to room temperature level, this was mixed with 66 ml of methanol and kept aside for one week. Buffer solution was prepared by adding 23.7 ml of 4gm

of sodium hydroxide diluted with 500 ml of water and 50 ml of 13.6 gm. of potassium dihydrogen phosphate diluted with 500 ml of water. 20 ml of this stock solution was diluted with water and made to 1000 ml to prepare the working buffer solution.

Procedure:

- Keep the slide in xylene for 15 minutes.
- Wash with graded alcohol
- Wash with water
- Add equal amounts of Giemsa stain and working buffer solution and cover the slide for five minutes.
- Wash with water for 2 minutes.
- Blot and air dry and then mount.

The organism *Helicobacter pylori* will appear purple in colour on Giemsa stain.

.

RESULTS

A total of 100 patients with laryngeal pathologies were enrolled in the study. They comprised of patients both with benign and malignant pathologies like vocal cord polyps, vocal cord keratosis, vocal cord papilloma, chronic laryngitis and vocal cord malignant pathology like carcinoma. Subjects with vocal cord polyps formed the majority of the study population comprising 57 %. Fig. 21 shows the distribution of the various vocal cord pathologies.

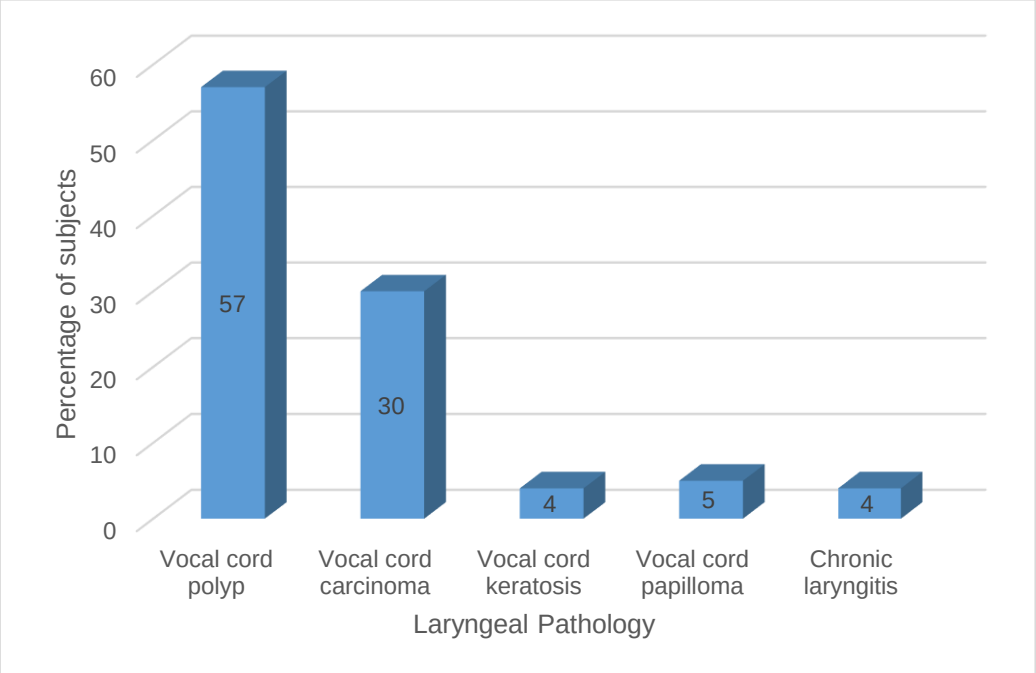


Fig. 21 Distribution of laryngeal pathologies.

Baseline characteristics of the study population

Age distribution:

The age of the patients in the study group ranged from 20 to 76 years with a mean age of 48 years. Majority of the patients were in the 40-60 age group. This indicates that the vocal cord lesions are present more frequently in the middle aged population than in the younger and elderly group. The age distribution of the study population is shown in Fig. 22

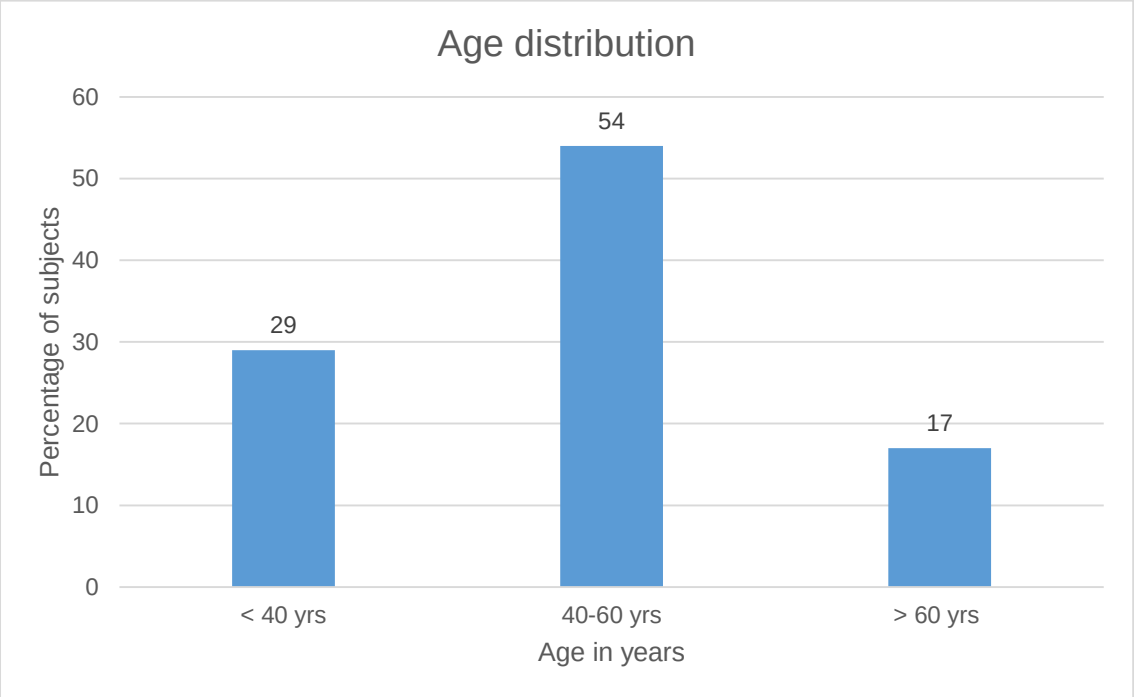


Fig. 22 Age distribution of the study population.

Sex distribution of the study population

Of the 100 subjects, 89% (89) were males and 11 % (11) were females. The sex distribution of the study subjects is shown in Fig. 23

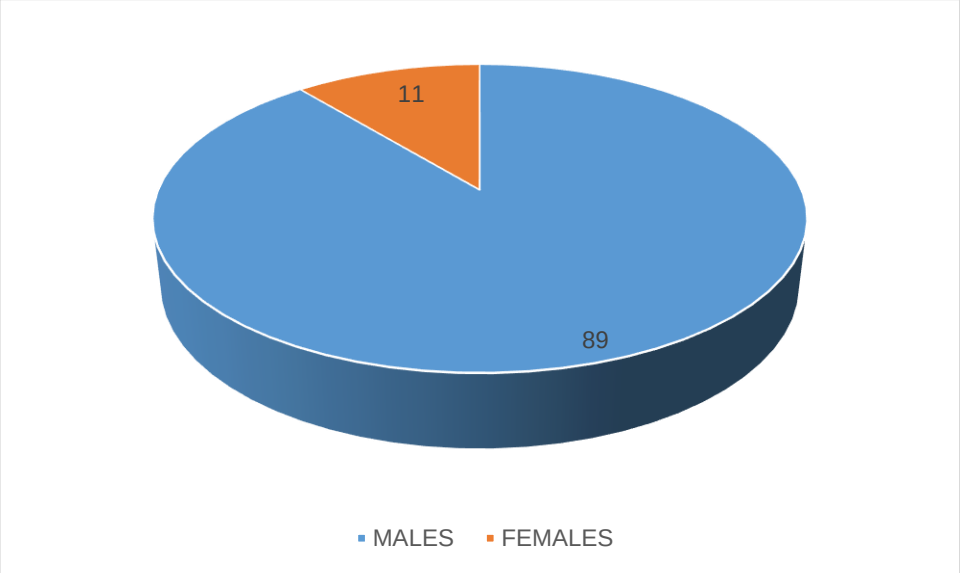


Fig. 23 Sex distribution of the study population.

Majority of the patients were Level 3 voice users (44%). Level 2 voice users comprised 27% of study population and 29 % were Level 4 voice users. There were no Level 1 voice users in the study. Fig. 24 shows distribution of the level of voice users. The study population included teachers, business men, policemen, engineers, farmers and housewives.

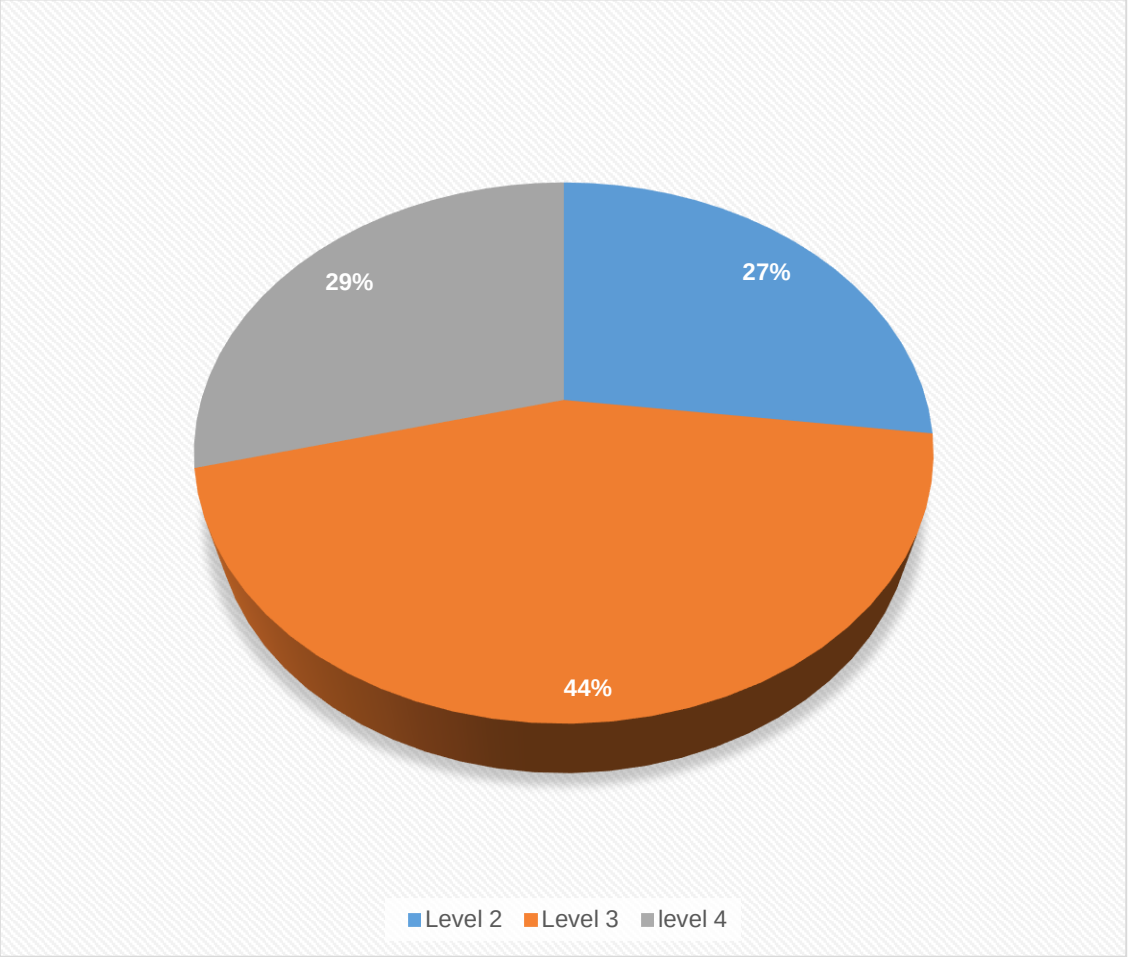


Fig. 24 Level of voice use in the study population.

The most common presenting symptom was hoarseness followed by throat clearing, foreign body sensation of throat and belching. Other symptoms were cough, difficulty in swallowing, difficulty in breathing and throat pain. The presenting symptoms of the study population is shown in Fig. 25

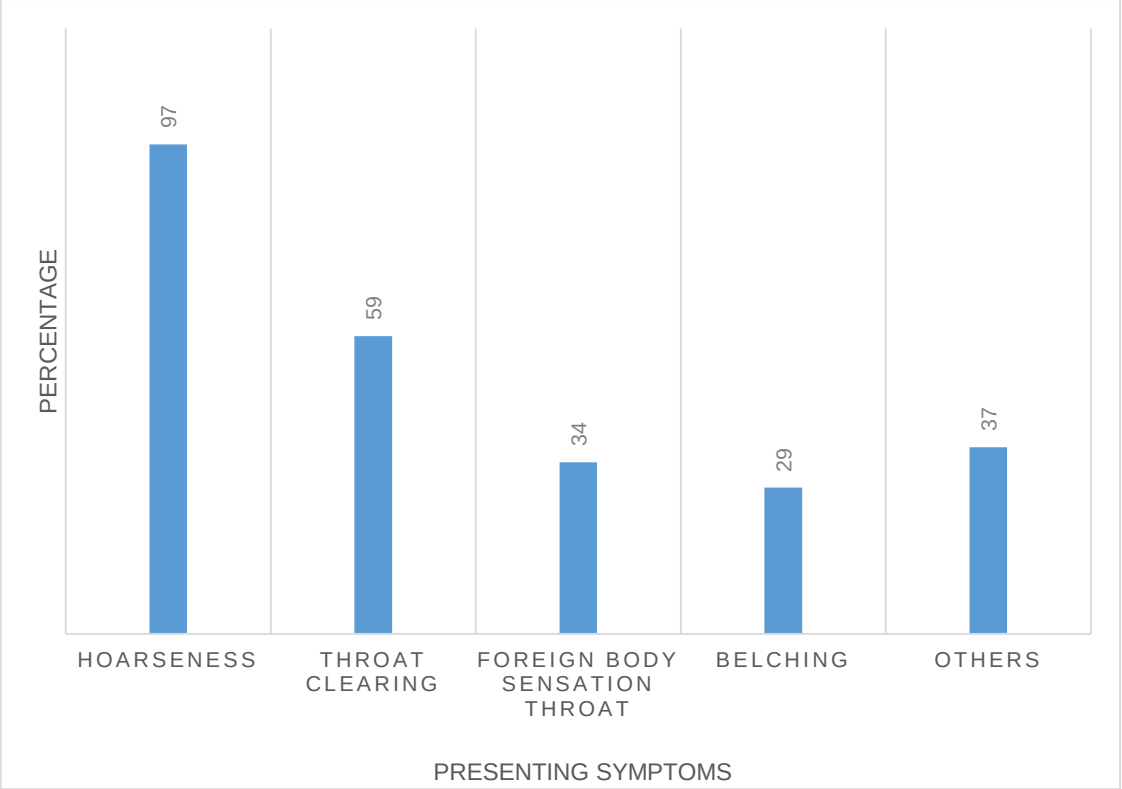


Fig. 25 Presenting symptoms of the study population.

Table 1. Presenting symptoms with the number of patients in the study.

PRESENTING COMPLAINTS	NUMBER OF PATIENTS
Hoarseness	97
Throat clearing	59
Foreign body sensation throat	34
Belching	29
Throat pain	8
Cough	13
Difficulty in breathing	11
Difficulty in swallowing	5
Neck swelling	0

Duration of hoarseness.

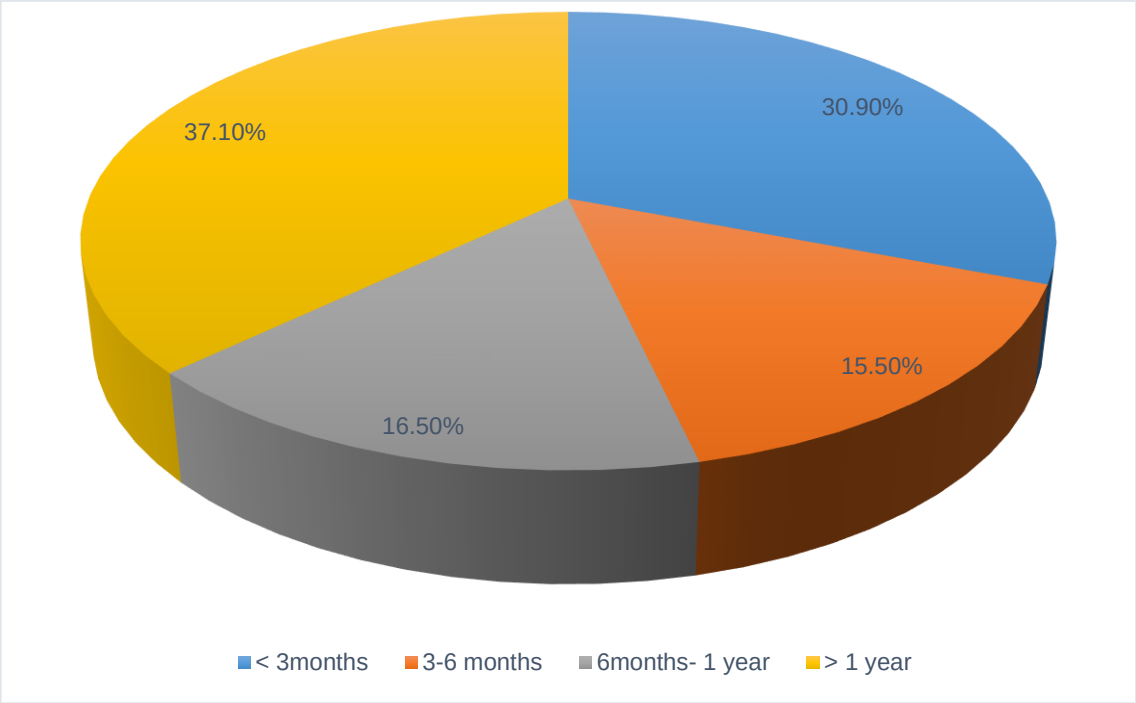


Fig. 26 Duration of hoarseness in the study population.

In our study, a total of 97 patients presented with hoarseness of voice. Out of this 37.1 % (36 patients) had this complaint for more than 1 year duration.

A total of 59 patients had history of voice abuse or voice misuse (Fig. 27). Out of this, 45.8 % reported voice abuse-misuse for more than 10 years and 33.9% had voice abuse-misuse for more than 5 years (Fig. 28). Thus voice abuse-misuse seemed to play a significant role in patients with vocal cord disorders.

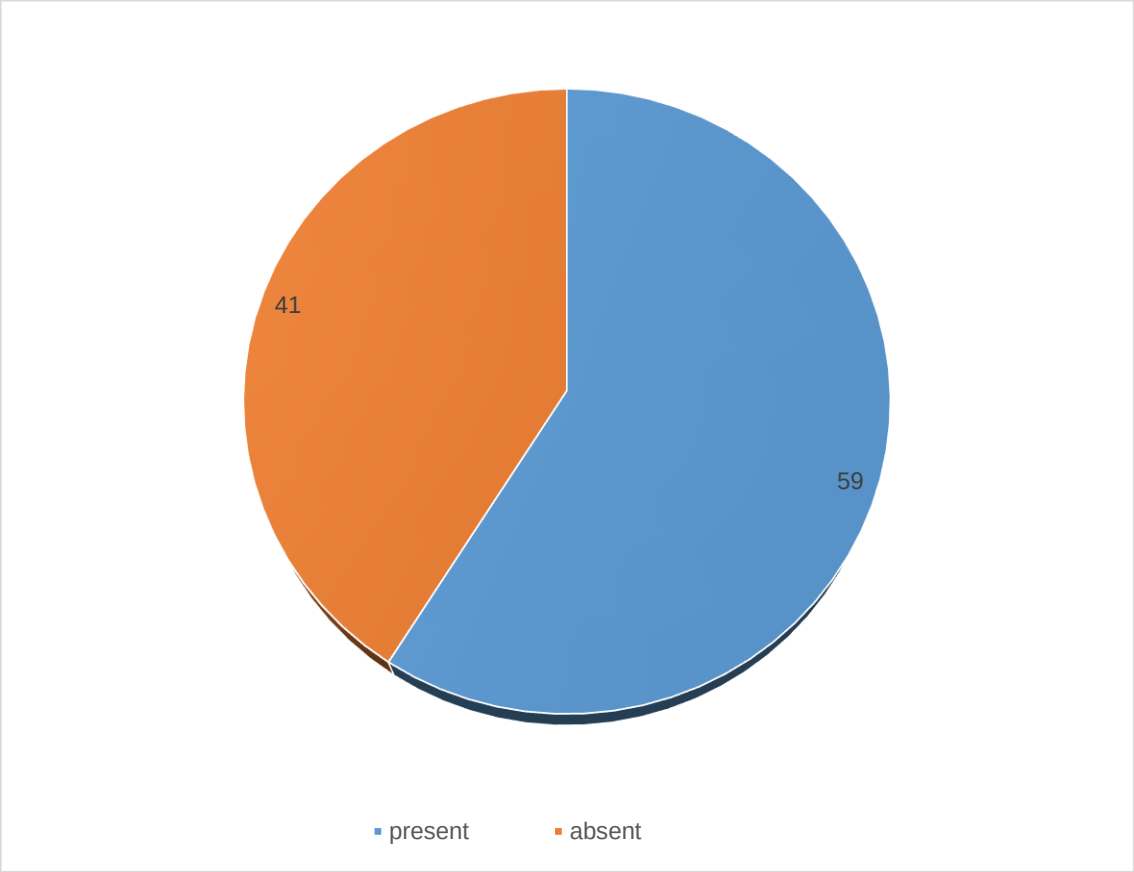


Fig. 27 Percentage of voice abuse-misuse in the study population.

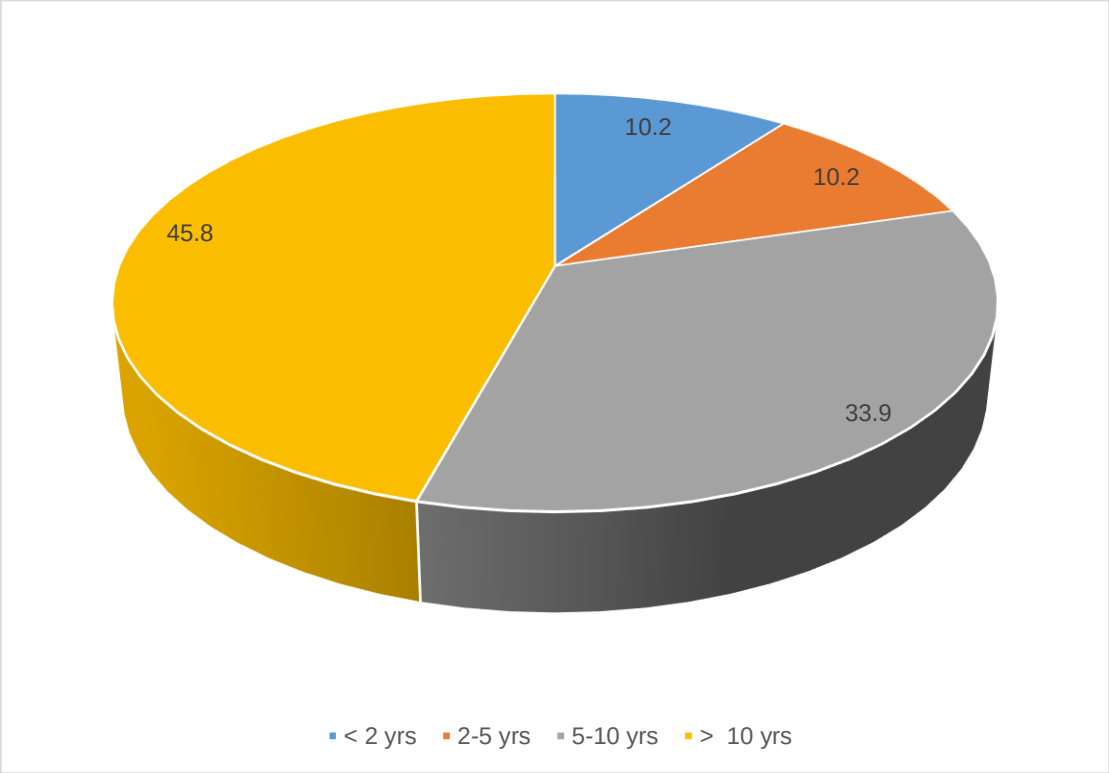


Fig. 28 Duration of voice abuse-misuse with the percentage of subjects in the study.

Risk factors in vocal cord pathologies.

The various risk factors analysed in our study were smoking, alcohol consumption and tobacco chewing.

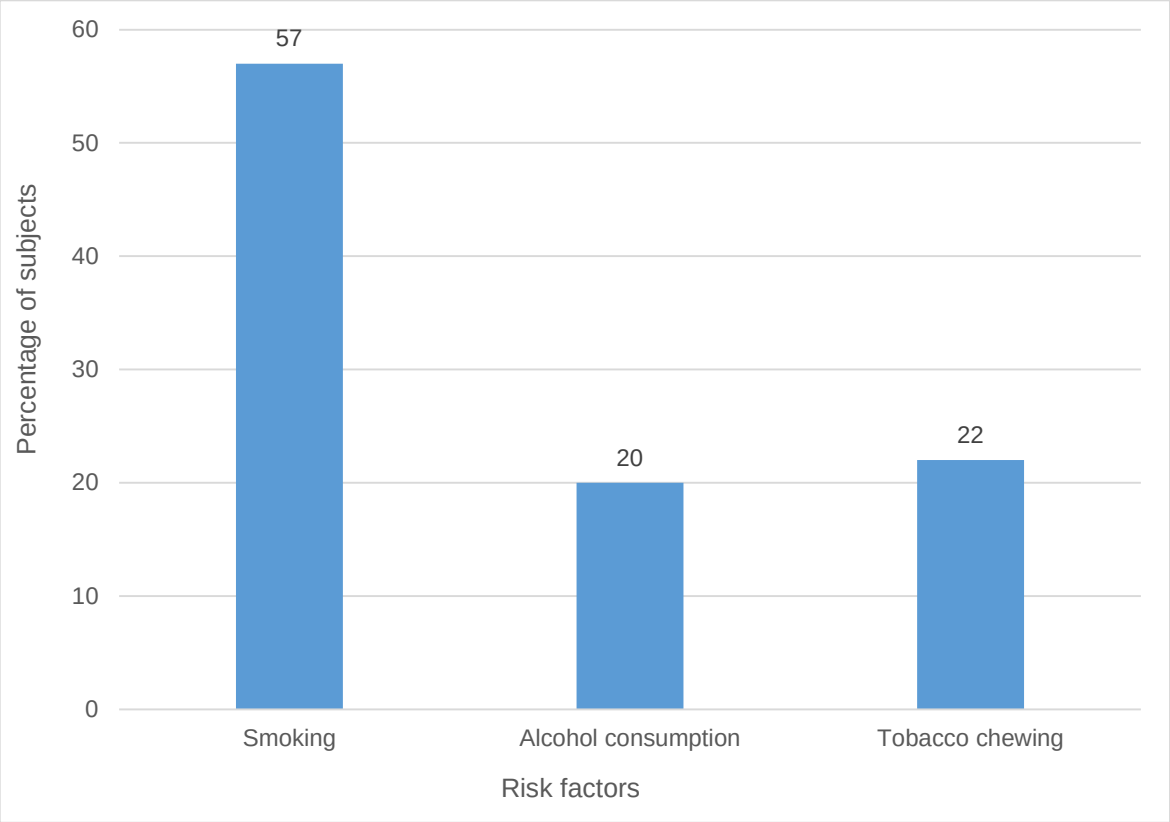


Fig. 29 Risk factors in the study population.

Majority of the patients smoked tobacco. The minimum duration of smoking was 2 years and the maximum was 55 years in the study. 20 patients had the habit of both smoking and alcohol consumption.

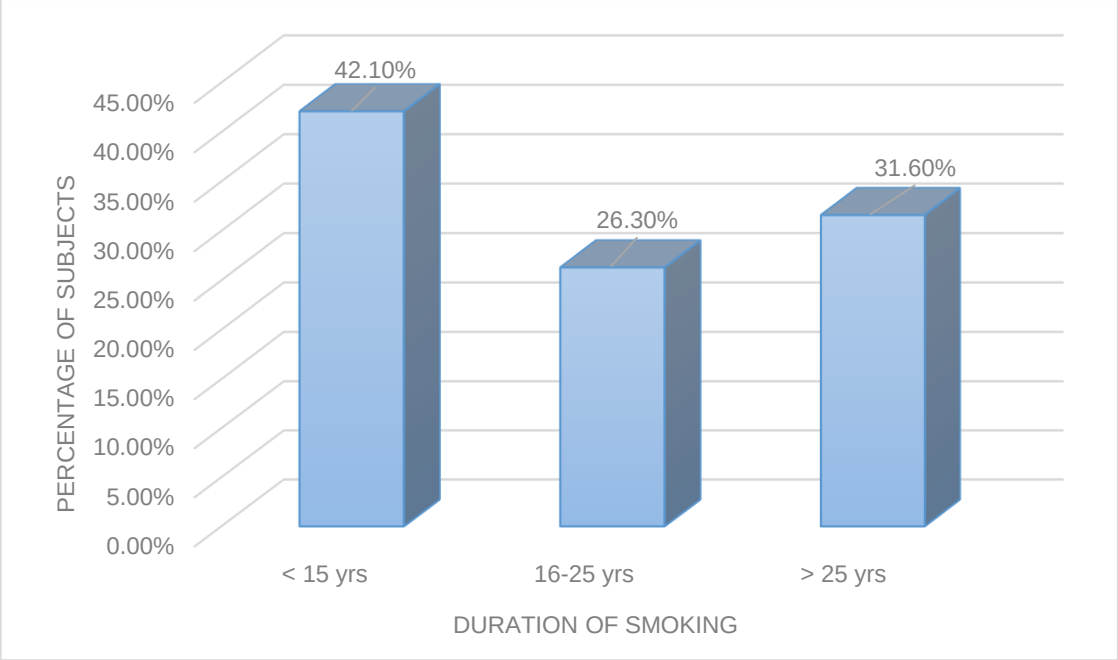


Fig. 30 Duration of smoking in the study population.

The prevalence of smokers in laryngeal cancer in our study was 80 %. Out of the 30 patients with laryngeal cancer, 24 were smokers. Fig. 31 shows the percentage of smoking in laryngeal cancer.

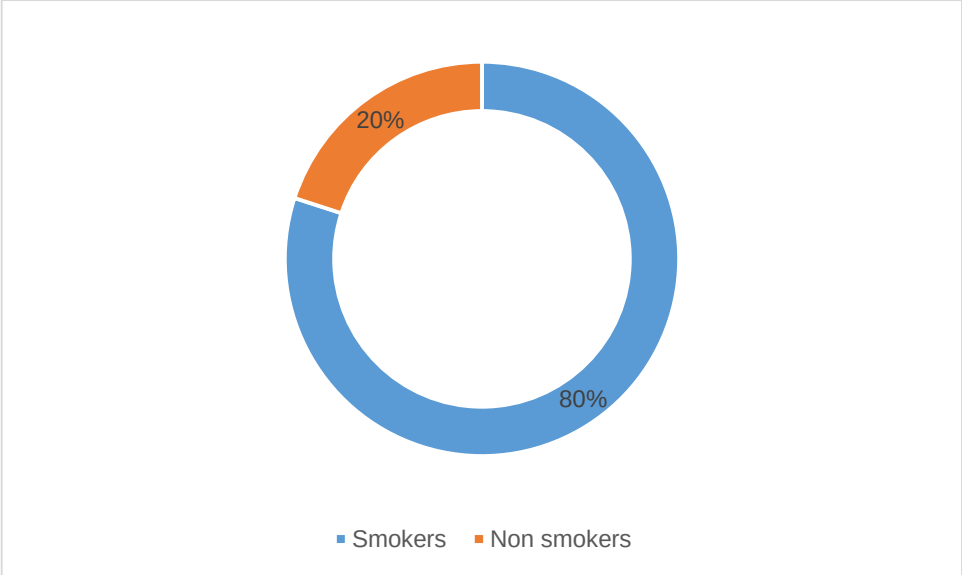


Fig. 31 Percentage of smokers in laryngeal cancer.

The percentage of alcohol consumption in subjects with laryngeal cancer was 20 %. 6 of the patients with laryngeal cancer were consumers of alcohol. The percentage of alcoholics were less compared to that of smokers.

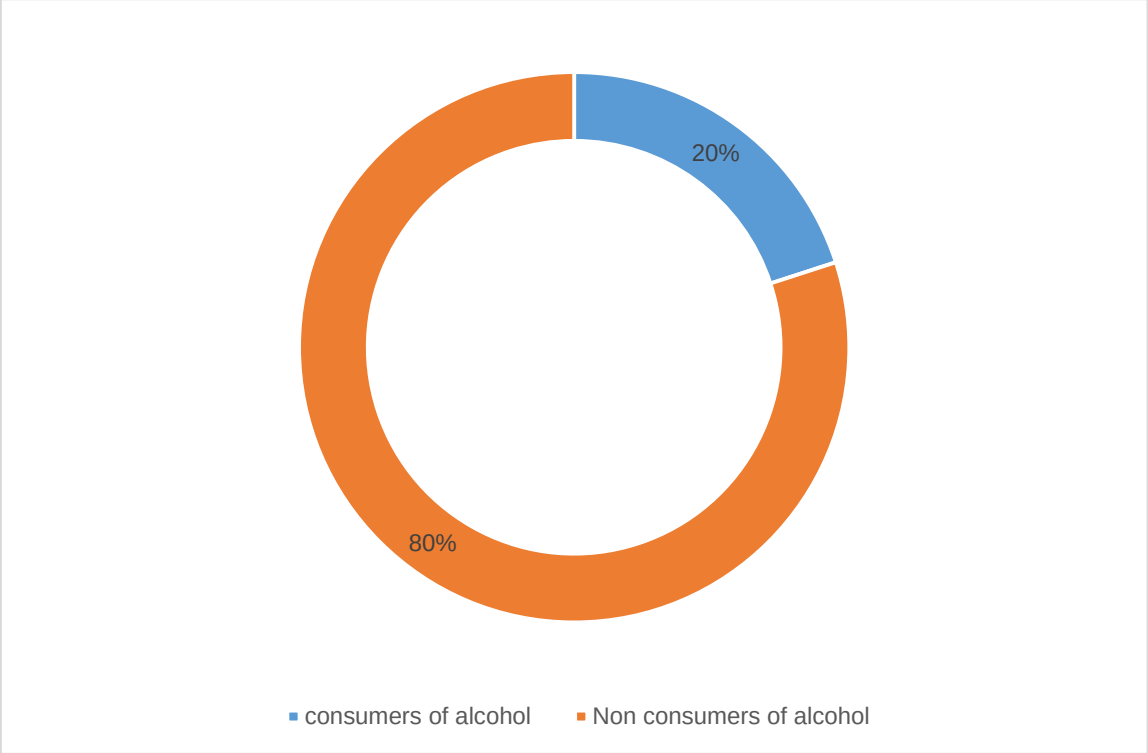


Fig. 32. Percentage of alcohol consumers in laryngeal cancer.

DETECTION OF HELICOBACTER PYLORI

PCR Analysis

Table 2. Results of Real time PCR analysis

LARYNGEAL PATHOLOGY	POSITIVE	NEGATIVE
Benign	0	100
Malignant	0	100

Giemsa Analysis

Table 3. Results of histopathological examination by Giemsa Staining

LARYNGEAL PATHOLOGY	POSITIVE	NEGATIVE
Benign	0	100
Malignant	0	100

Out of the 100 cases studied, none of them were positive for *Helicobacter pylori* by PCR analysis or Giemsa staining method.

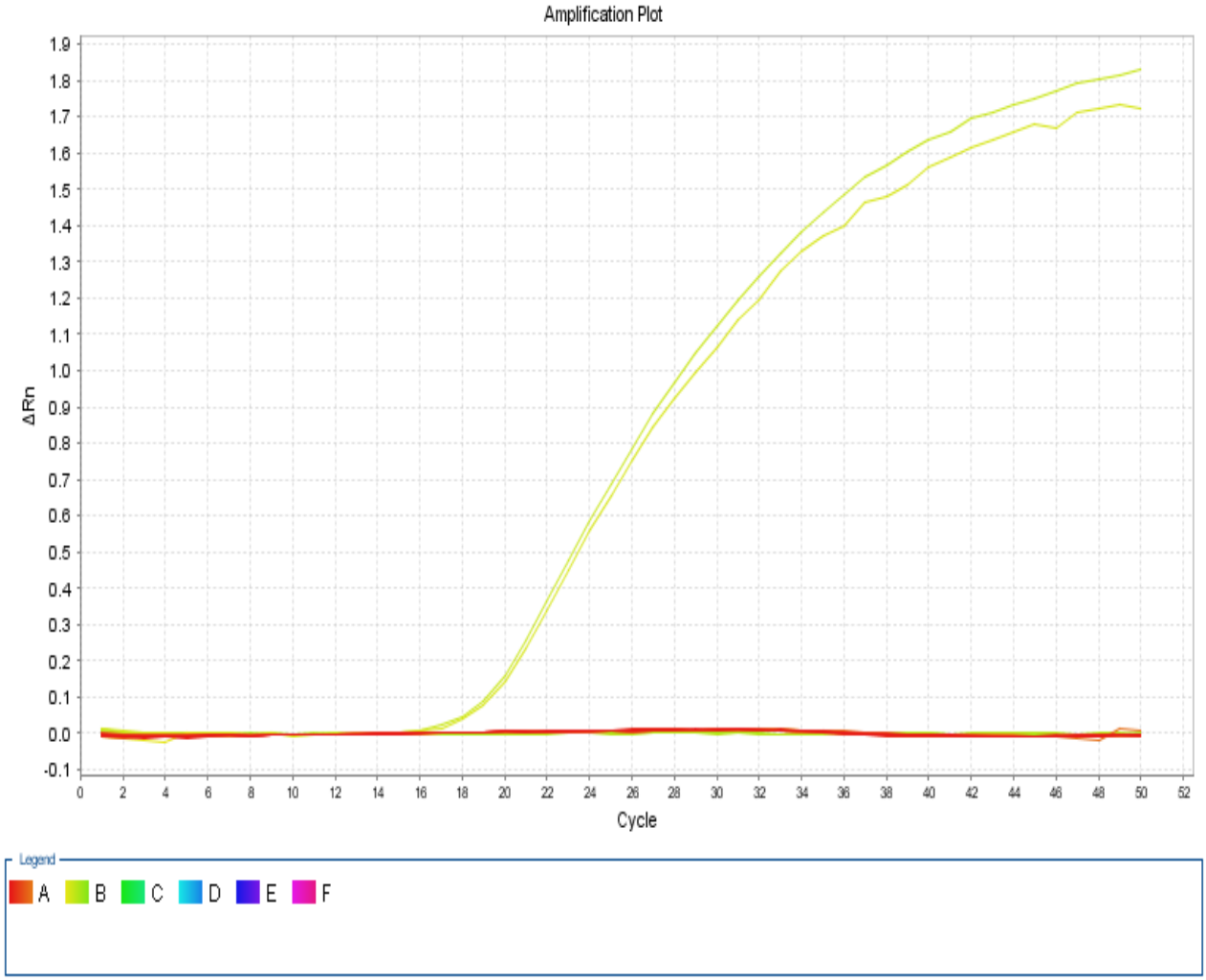


Fig. 33 Results of real time PCR analysis

The sigmoid shaped green curve indicates the positive control. The red coloured line indicated the study cases which were negative.

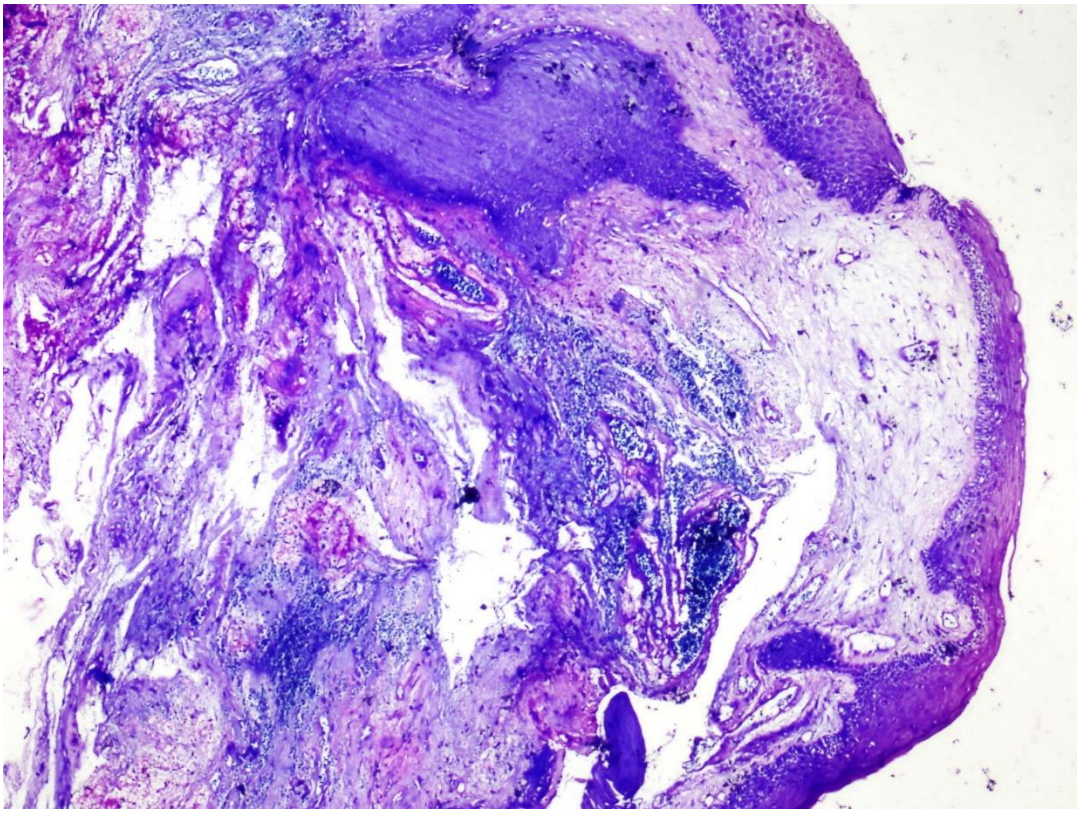


Fig. 34 Giemsa staining on vocal cord polyp

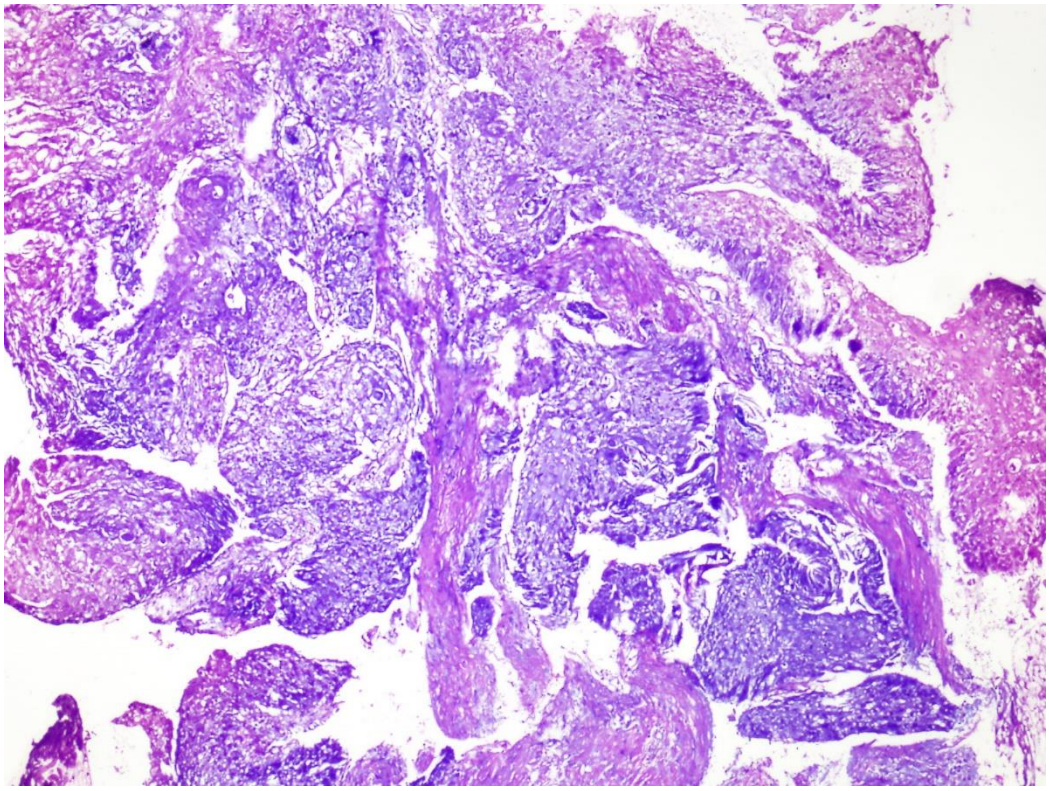


Fig. 35 Giemsa staining on carcinoma larynx.

Laryngopharyngeal reflux and laryngeal pathology

We also analysed the prevalence of laryngopharyngeal reflux in these subjects. Out of 100 patients, 21 had complaints of belching and acid reflux. The rest of the study subjects had complaints of foreign body sensation throat, frequent throat clearing etc.

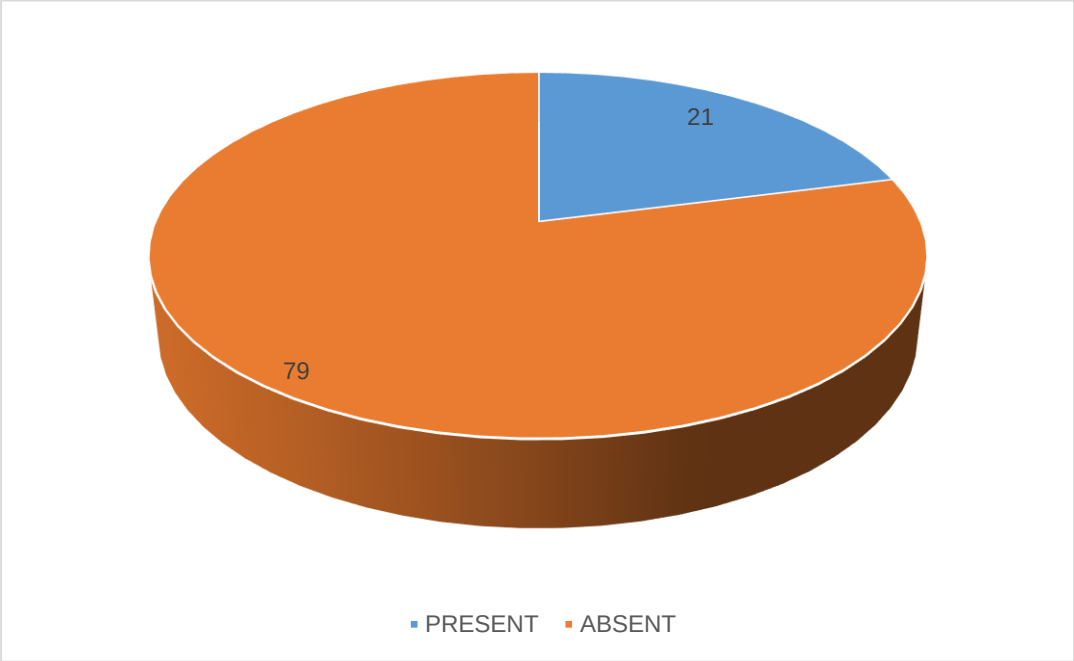


Fig. 36 Belching in the study population

Presence of laryngopharyngeal reflux was assessed by Reflux Symptom Index Questionnaire and Reflux Finding Score.

Out of the total 100 patients, 14 patients had a significant RSI score. RSI score more than or equal to 13 was considered to be significant. Out of these 14 patients, 10 had vocal cord polyp and 4 had carcinoma larynx

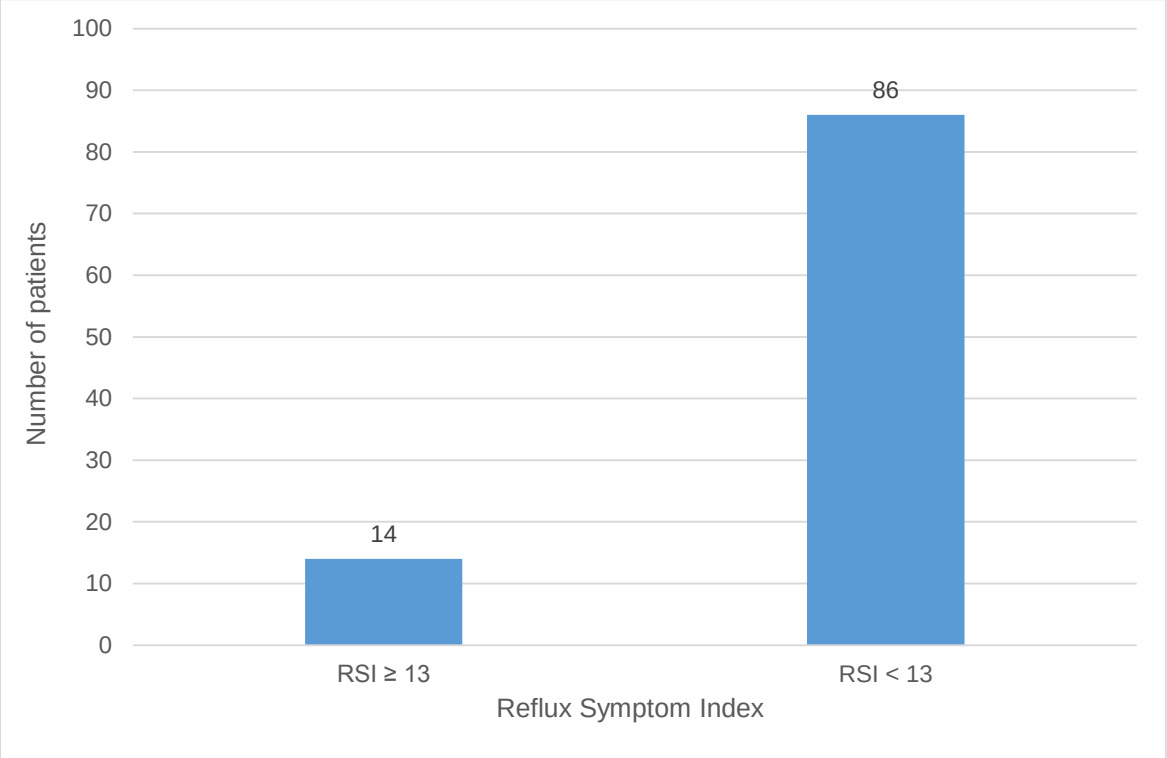


Fig. 37 Reflux Symptom Index in the study population.

Out of the 100 patients, 35 had a significant Reflux Finding Score. 21 of these patients had vocal cord polyp. Reflux Finding Score more than or equal to 7 was considered to be significant.

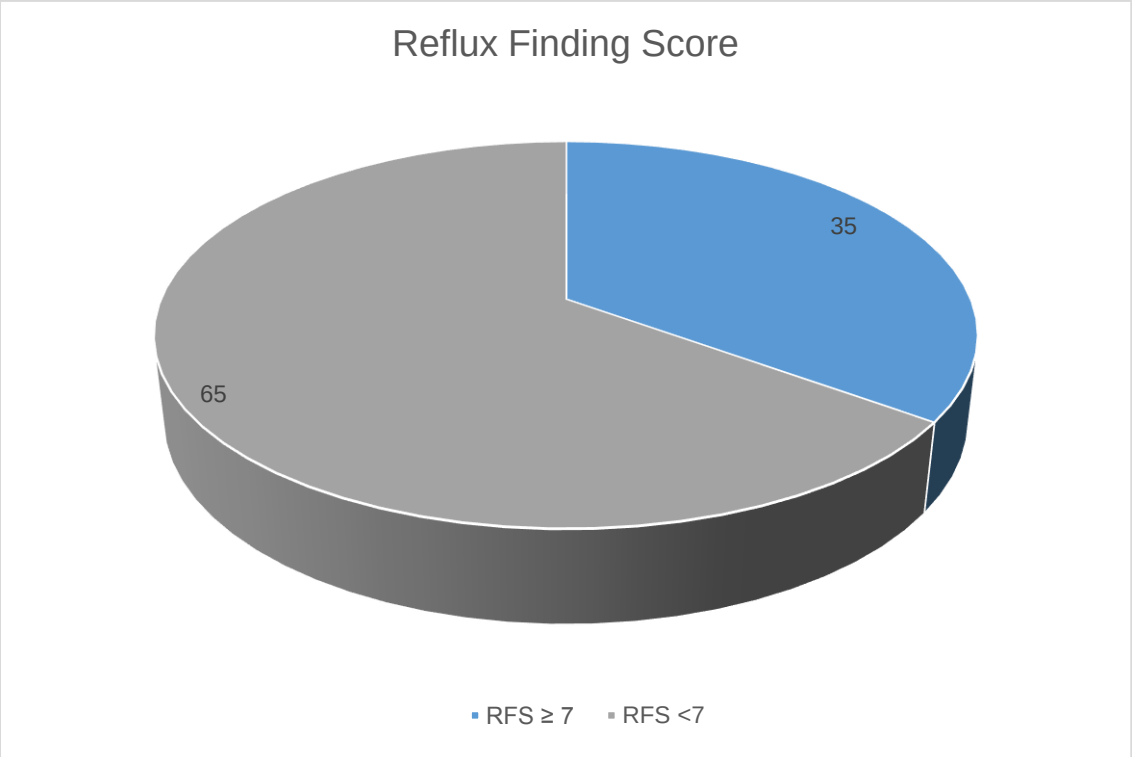


Fig. 38 Reflux Finding Score in the study population.

Reflux Symptom Index versus Reflux Finding Score.

On comparing RSI with RFS, it was noted that out of the 14 patients who had laryngopharyngeal reflux as per RSI, 10 (71.4%) patients also had laryngopharyngeal reflux as per RFS.

Table 4. Comparison of RSI with RFS

Reflux Finding Score	Reflux Symptom Index	
	Negative	Positive
Negative	65	4
Positive	25	10

The correlation between RSI and RFS was found to be statistically significant with a p value of .002.

We also analysed the relationship between dinnertime and bedtime gap and significance of reflux. A gap of less than 2 hour between dinnertime and bedtime is associated with increased reflux. In our study, a total of 90 patients had less than 2 hour interval between dinner time and bedtime. Out of this 15.6% (n=14) had significant RSI and 35.6% (n=32) had significant RFS. Out of the 10 patients who had dinnertime and bedtime gap more than 2 hours, none of them had significant RSI and only 3 of them (35%) had significant RFS.

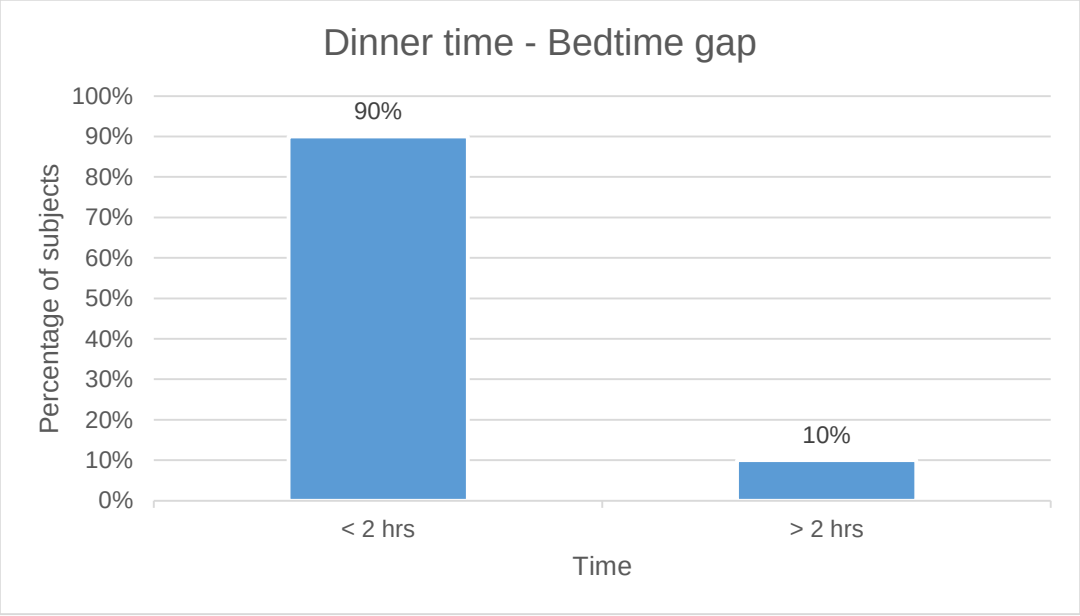


Fig. 39 Dinnertime bedtime gap and percentage of subjects.

Out of the total 57 patients with vocal cord polyps, 36.8% (n=21) had significant reflux finding score and in 63.2% this was not significant and the p value was not significant.

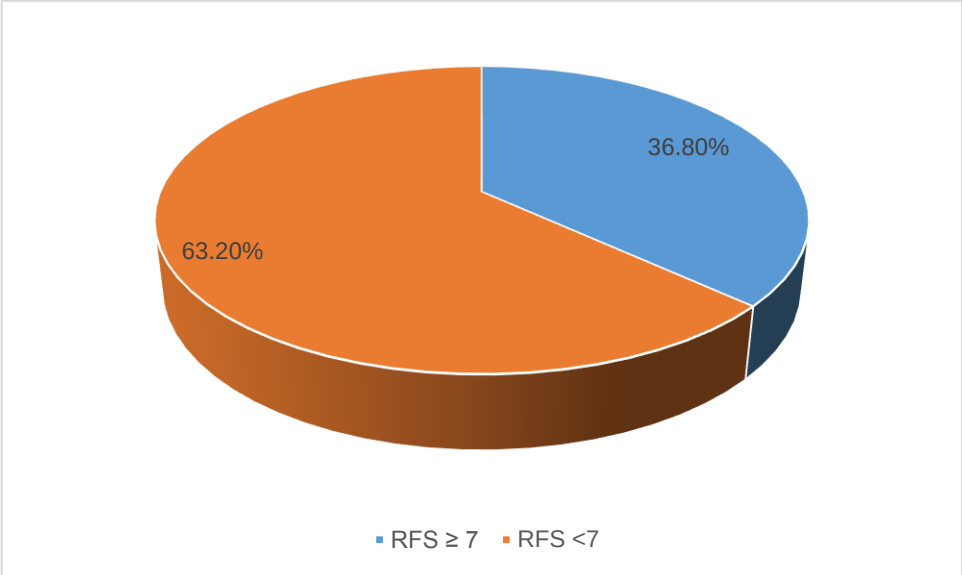


Fig. 40 Vocal cord polyps and Reflux Finding Score

Out of the total 30 patients with carcinoma of larynx, 36.7 % (n=11) had significant RFS and in 63.3% (n =19) it was not significant.

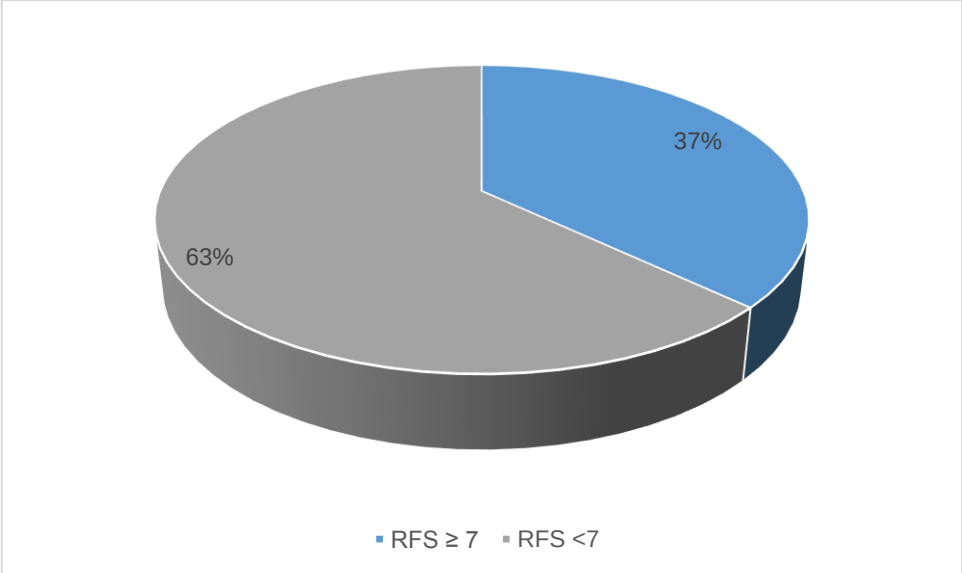


Fig. 41 Vocal cord carcinoma and Reflux Finding Score.

DISCUSSION

Helicobacter pylori discovered in 1982 by Barry Marshall and Robin Warren is a gram negative spiral shaped microaerophilic bacteria known to colonise the gastric epithelial cell surface. Whether the bacteria can colonise the upper aerodigestive tract in areas like the oral cavity, pharynx and larynx is still being studied. This prospective study was undertaken to study if the bacteria was present in the laryngeal pathological tissue. Also the association of laryngopharyngeal reflux and laryngeal pathologies was studied along with its association in patients in whom *H. pylori* was detected. *Helicobacter pylori* is a common coloniser of human gastric mucosa. It reaches the stomach which is its primary reservoir via the oral cavity. The transmission can be either oro-oral or faeco-oral. Whether the bacteria can colonise in the upper aerodigestive tract involving oral cavity, pharynx and larynx are still under debate. Mapestone et al (3) demonstrated the presence of *H. pylori* in the dental plaque and saliva of patients with proven gastric colonisation by means of nested PCR. Kusano et al (2) have demonstrated the presence of coccoid forms of *Helicobacter pylori* in the tonsillectomy specimens of patients with Ig A nephropathy by means of immunofluorescence and immunoelectron microscopy, however they could not culture the bacteria .

Pajic´-Penavic´ et al (4) studied normal laryngeal tissue for the presence of *H. pylori* and concluded that it was not a commensal there. Recently various studies were carried out to determine whether this bacterium could be responsible for pathogenesis of laryngeal diseases and whether the eradication of the bacteria would help in the treatment of

the same. Larynx, an area of upper aerodigestive tract is a potential area that could be exposed to the gastric contents by means of reflux. Since *Helicobacter pylori* infection is an established aetiological agent for chronic gastritis and gastric cancer, it could be postulated that the organism might play a role in the pathogenesis of laryngeal disease in general and laryngeal cancer in particular. *Helicobacter pylori* is the first bacterium to be labelled as a definite carcinogen for gastric cancer and mucosa associated lymphoma of the stomach by the World Health Organisation (26).

Studies have been conducted to determine the role of *Helicobacter pylori* in laryngeal diseases. Akbayir et al (76) studied the presence of *H. pylori* in 50 patients of laryngeal cancer who underwent total or partial laryngectomy and in 50 patients with benign laryngeal pathologies by histopathological and immunohistochemical methods, but none of them were positive for *H. pylori.* Masoud et al (77) investigated the role of *H. pylori* in 44 patients with squamous cell carcinoma of larynx and 30 patients with benign laryngeal lesions like polyp, nodule and granuloma by means of rapid urease testing and routine histopathological examination. None of the tests could detect *H. pylori* in any of these specimens. They concluded that *H. pylori* is unlikely to colonise larynx and that larynx is not a permanent reservoir for this bacterium. Our study used the most specific method of detection, i.e. real time PCR and did not detect the presence of bacterium in any of the benign or malignant laryngeal lesions.

Some studies have shown a positive association of *Helicobacter pylori* in laryngeal pathologies. Fellman et al (7) studied the presence of *H. pylori* in the upper aerodigestive tract of patients with proven gastric colonisation and established their presence in the larynx, pharynx and oral cavity by means of PCR analysis. They concluded that the upper

aerodigestive tract can be an additional reservoir for this bacterium in patients with *H. pylori* gastritis. Rubin et al (78) and Borowski et al (79) noted a positive association between *H. pylori* and chronic laryngitis whereas Jaspersen et al (80) proved a negative association. Rubin et al tested for the presence of serum antibodies for *H. pylori* in patients with laryngeal lesions whereas Borowski et al and Jaspersen et al performed rapid urease testing for detecting the bacteria. Also Ozyurt et al (26) detected *H. pylori* DNA in laryngeal pathologies, nasal mucosa and nasal polyps by real time PCR method. A similar study was conducted by Siupsinskiene et al (81) to detect the presence of *H. pylori* in patients suffering from benign laryngeal diseases and laryngeal cancer. They performed rapid urease testing and histopathological examination of the laryngeal specimen by modified Giemsa technique for identification of the bacterium and could identify the bacterium in 45.5% of patients with chronic laryngitis and 46.2% of patients with laryngeal cancer. They concluded that *H. pylori* infection could be a possible risk factor for laryngeal pathologies. Cekin et al (74) also studied the association of *Helicobacter pylori* and laryngopharyngeal reflux in patients with laryngeal pathologies. They performed real time PCR to detect *UreC* gene in *H. pylori* and the presence of laryngopharyngeal reflux was assessed by Reflux Symptom Index and Reflux Finding Score similar to our study. They could detect *H. pylori* in 55.8% of subjects with laryngeal pathologies. 69.8% of subjects had significant Reflux Finding Score, but they could not find a significant association between reflux and *H. pylori* positivity.

In our study we used the Genesig kit which detects the target *ureA* gene in *H. pylori*. The targets routinely used to detect *H. pylori* by PCR methods include the 16S rRNA gene, the random chromosome sequence, the 26-kDa species-specific antigen (SSA) genes, the urease (*ureA*) gene and the *glmM (ureC)* gene. Espinoza et al (82) compared the efficacy of *ureA* gene and *ureC* gene in detecting *H. pylori* by PCR method and concluded that PCR assay

using *ureC* gene could detect all those strains which were not picked up by *ureA* analysis. The *ureC* gene is a house keeping gene which directly participates in the cell wall synthesis of the bacteria and is unrelated to urease production unlike the *ureA* gene. Another study conducted by Smith et al (83) to evaluate the most appropriate method of PCR to detect *H. pylori* showed that detection using *ureA* gene had poor specificity and that using 26 kDa was the best method. Since our study used only one target to detect *H. pylori* we could have possibly missed out the organism by PCR analysis.

Giemsa staining could not detect any *Helicobacter pylori* in both benign and malignant lesions in our study. This can be explained by a probable low bacterial load in the larynx. In such cases Giemsa staining has less sensitivity to detect the organism. Also it is said that multiple biopsies from different sites increases the chance of detecting the organism which was not possible in our study since most of the laryngeal lesions were only few millimetres in size. *H. pylori* in biopsy slides appear as a comma shaped or S- shaped bacillus 2.5- 4 μm long and 0.5- 1 μm thick. Even for detection of the bacteria in the gastric antrum, a good quality biopsy specimen with adequate epithelial cells are necessary and it is difficult to draw a conclusion when the bacterial load is very low with altered morphology (84). In one another study, Aslan et al (85) demonstrated the presence of *H. pylori* in tonsillar specimen by PontoDry test but failed to demonstrate it in the histopathological specimen by haematoxylin-eosin, Giemsa or Warthin Starry silver staining technique.

In our study of total 100 patients, a definite male preponderance was seen. 89 % of the study population consisted of men. This is in favour of male predominance seen in vocal cord pathologies as described by various other studies. Banjara et al (86) analysed the demographic characteristics of various vocal cord pathologies and concluded that most of

them occurred in male population. Majority of the patients in the study belonged to the middle aged group. Also laryngeal cancers showed a definite male preponderance as proven by previous studies (87). This is because men used to smoke more and consume alcohol more than women. In our study, out of 30 patients with laryngeal cancer, 28 were males.

The various risk factors analysed in our study were smoking, alcohol consumption and tobacco chewing. Smoking was reported in 58% of the study population and is regarded as one of the major risk factors for vocal cord lesions, especially malignancies of the vocal cord. Out of the 30 patients with carcinoma of larynx, 24 had history of smoking. Thus the prevalence of smoking in carcinoma in our study was 80 %. This was in keeping with various other studies described in literature (88,89). Larynx is more susceptible to the harmful effects of smoking as compared to other areas in head and neck. This can be explained by the aerodynamics of the respiratory airflow as the airflow becomes more turbulent in larynx resulting in higher exposure to the inhaled carcinogens. Alcohol consumption did not show a significant role in laryngeal cancers in our study. The prevalence of alcohol consumption in patients with laryngeal cancer was only 20%.

Vocal abuse or voice misuse is another risk factor for vocal cord pathologies. Hoarseness was the predominant symptom in the study group. Out of 100 patients, 97 presented with change in voice. Voice abuse leads to phono trauma and is the most important factor underlying the development of benign vocal cord pathologies. Phonotrauma refers to the physical stress on the tissues of vocal fold that occur during phonation (90). Phonotrauma depends on the amount and intensity of voice use. In our study, 59% of the population had history of voice abuse and among them majority (88.9%) presented with vocal cord polyps.

Laryngopharyngeal reflux is a common diagnosis made by otolaryngologists in patients presenting with hoarseness. LPR manifests in a wide variety of symptoms as globus pharyngeus, hoarseness, sore throat, dysphagia, throat clearing or chronic cough. So it is very important to make an accurate diagnosis. Double probe pH manometry is cumbersome to be performed in every patients who presents with symptoms of reflux because it is invasive and costly. To overcome this difficulty endoscopic examination of laryngopharynx for signs of reflux by Reflux Finding Score was introduced by Belafsky.

Laryngeal mucosa is poorly suited to resist the components of reflux as compared to oesophageal mucosa. As many as fifty reflux episodes in a day is considered to be physiological in oesophagus whereas a single episode of reflux into the laryngopharynx is detrimental. Pepsin in the refluxate is believed to damage the cell membrane integrity by disrupting the intercellular junction complex and increasing the intercellular space. Also acid remains in contact with the mucosa for a prolonged period of time due to lack of peristalsis in the larynx as compared to oesophagus. Since the laryngeal mucosa does not come in contact with saliva normally, the acid neutralisation power of salivary bicarbonate is also not effective. The mucosal damage once occurred takes at least six months to heal. If the inflammation goes on the symptoms increases and the severity of mucosal damage increases (91).

We also analysed the presence of reflux by means of Reflux Symptom Index and Reflux Finding Score. In our study, a total of 35 patients had significant reflux finding score and 14 patients had significant Reflux Symptom Index. Congested arytenoids was the most common finding observed followed by posterior commissure hypertrophy. In a study conducted by Koufman et al (92) the prevalence of reflux in patients with laryngeal disorders was assessed by double probe pH monitoring and was estimated to be around 50% in the study

population. They concluded that reflux plays a significant role in patients with laryngeal pathologies. In our study, out of the total 57 patients with vocal cord polyps, 36.8% (n=21) had significant reflux finding score. Our study also found an association between reflux and vocal fold polyps but this was not statistically significant.

The association of laryngopharyngeal reflux in laryngeal cancer is widely being studied, but is the association causal is yet unclear. In the last few years reflux has been studied as an independent carcinogen as well as a cocarcinogen along with smoking and alcohol consumption in laryngeal cancer. Copper et al (93) performed double probe pH monitoring in 24 patients with squamous cell carcinoma of larynx and pharynx and concluded that 16 of these patients had pathological reflux. Refluxed acid and bile irritates the laryngeal mucosa leading to chronic laryngitis and in the long run to laryngeal carcinoma. We studied the significance of reflux in patients with laryngeal cancer by Reflux finding score and found out that 11 (36.7%) out of 30 patients had reflux which was not statistically significant.

LIMITATIONS

Our study was also not free from limitations. We used the Genesig kit for detecting *UreA* gene in *H. pylori* by real time PCR method.

The amount of DNA in the test specimen was variable. The DNA tested was not pure bacterial DNA. Since the sample tested was a tissue specimen, it had a mixture of host DNA with the bacterial DNA. Hence the amount of bacterial DNA if present might have been very minimal or might not be present in the sample tested. Since laryngeal biopsy specimens are very small, the presence of bacteria in the sample tested is questionable.

For effective detection of DNA template by PCR, the minimum amount of DNA required is about 10-20ng/µl. At least 5ng of DNA template is required for effective detection as per the kit instruction. DNA present below the estimated threshold hinders the detection of target. Only few samples in our study had this required nucleic acid concentration. Also the RNA to DNA ratio in the samples analysed were not showing convincing values. Thus the amount of sample available and the methods of DNA extraction is a definite limitation of our study.

Another limitation of our study was determination of Reflux Finding Score. This was a very subjective study and was done by different doctors in the endoscopy room. So we might have missed out some patients who really had reflux.

One of our exclusion criteria was that the patient should not be on any proton pump inhibitors for at least a period of 4 weeks. Since this a very common drug taken by people, some of them might have actually taken this medication and were not aware of the same.

CONCLUSION

In this study conducted on 100 patients with hoarseness and laryngeal pathologies, *Helicobacter pylori* was not isolated in the laryngeal tissue biopsy material suggesting that the organism had no role in the development of benign or malignant laryngeal pathology. PCR test using primers which can detect all the genes (*UreA, glmM*, 26kDa SSA, 16S rRNA) in *H. pylori* would be more specific than detecting a single gene which might possibly be missed during testing.

Our study also found an association between laryngopharyngeal reflux in laryngeal pathologies, even though the association did not reach statistical significance.

BIBLIOGRAPHY

1. Franceschi F, Gasbarrini A. Helicobacter pylori and extragastric diseases. *Best Pract Res Clin Gastroentero*l. 2007;21(2):325–34.

2. Kusano K, Inokuchi A, Fujimoto K, Miyamoto H, Tokunaga O, Kuratomi Y, et al. Coccoid Helicobacter pylori exists in the palatine tonsils of patients with IgA nephropathy. *J Gastroenterol*. 2010 Apr;45(4):406–12.

3. Mapstone NP, Lynch DA, Lewis FA, Axon AT, Tompkins DS, Dixon MF, et al. Identification of Helicobacter pylori DNA in the mouths and stomachs of patients with gastritis using PCR. *J Clin Pathol.* 1993 Jun;46(6):540–3.

4. Pajić-Penavić I, Danić D, Maslovara S, Gall-Trošelj K. Absence of Helicobacter pylori in healthy laryngeal mucosa. *J Laryngol Otol.* 2012 Feb;126(2):196–9.

5. Pirzadeh A, Doustmohammadian N, Khoshbaten M, Doustmohammadion S. Is there any association between Helicobacter Pylori infection and laryngeal carcinoma? *Asian Pac J Cancer Prev*. 2011;12(4):897–900.

6. Pilotto A, Franceschi M. Helicobacter pylori infection in older people. *World J. Gastroenterol*. 2014 Jun 7;20(21):6364–73.

7. Fellmann J, Weisert JU, Soltermann A, Morand G, Morra L, Moch H, et al. Helicobacter pylori detected in pharyngeal and laryngeal pathologies in patients with proven gastric colonization. *Head Neck*. 2013 Aug 30;

8. Saki N, Samarbaf Zadeh AR, Sheikhpour Jonaky R, Noori SM, Kayedani GA, Nikakhlagh S. The Prevalence Rate of Helicobacter pylori Infection in, Chronic Otitis Media With Effusion Patients. *Jundishapur J Microbiol*. 2014 Mar;7(3):e15694.

9. Medical Microbiology, 18th Edition | David Greenwood, Richard Slack, Michael Barer, Will Irving | ISBN 9780702040894 [Internet]. [cited 2013 Dec 12]. Available from: http://store.elsevier.com/Medical-Microbiology/isbn-9780702040894

10. Zanotti G, Cendron L. Structural and functional aspects of the Helicobacter pylori secretome. *World J. Gastroenterol*. 2014 Feb 14;20(6):1402–23.

11. Xiang Z, Censini S, Bayeli PF, Telford JL, Figura N, Rappuoli R, et al. Analysis of expression of CagA and VacA virulence factors in 43 strains of Helicobacter pylori reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. *Infect Immun.* 1995 Jan;63(1):94–8.

12. Chambers MG, Pyburn TM, Gonzalez-Rivera C, Collier SE, Eli I, Yip CK, et al. Structural Analysis of the Oligomeric States of Helicobacter pylori VacA Toxin. *J Mol Biol.* 2013 Feb 8;425(3):524–35.

13. Fang T-J, Lee L-A, Li H-Y, Yang C, Huang C-G. Helicobacter pylori colonization in the larynges of patients with hoarseness. *The Laryngoscope*. 2008 Mar;118(3):389–93.

14. Wang XM, Yee KC, Hazeki-Taylor N, Li J, Fu HY, Huang ML, et al. Oral Helicobacter pylori, its relationship to successful eradication of gastric H. pylori and saliva culture confirmation. *J Physiol Pharmacol*. 2014 Aug;65(4):559–66.

15. Vaishnavi C. Infections of the Gastrointestinal System. 1 edition. New Delhi: Jp Medical Ltd; 2013. 621 p.

16. Hagymasi K, Tulassay Z. Helicobacter pylori infection: New pathogenetic and clinical aspects. *World J. Gastroenterol* . 2014 Jun 7;20(21):6386–99.

17. Souza RCA de, Lima JHC. Helicobacter pylori and gastroesophageal reflux disease: a review of this intriguing relationship. *Dis Esophagus*. 2009;22(3):256–63.

18. Ghoshal UC, Chourasia D. Gastroesophageal Reflux Disease and Helicobacter pylori: What May Be the Relationship? *J Neurogastroenterol Motil*. 2010 Jul;16(3):243–50.

19. Polat FR, Polat S. The effect of Helicobacter pylori on gastroesophageal reflux disease. *JSLS*. 2012 Jun;16(2):260–3.

20. Yılmaz T, Bajin MD, Günaydın RÖ, Ozer S, Sözen T. Laryngopharyngeal reflux and Helicobacter pylori. *World J. Gastroenterol*. 2014 Jul 21;20(27):8964–70.

21. Howden CW, Hunt RH. Guidelines for the management of Helicobacter pylori infection. Ad Hoc Committee on Practice Parameters of the American College of Gastroenterology. *Am J Gastroenterol.* 1998 Dec;93(12):2330–8.

22. Hahn M, Fennerty MB, Corless CL, Magaret N, Lieberman DA, Faigel DO. Noninvasive tests as a substitute for histology in the diagnosis of Helicobacter pylori infection. *Gastrointest Endosc.* 2000 Jul;52(1):20–6.

23. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of Helicobacter pylori: comparison of staining methods. *J Clin Pathol.* 2000 Oct;53(10):756–9.

24. Real Time PCR Handbook [Internet]. [cited 2014 Jun 24]. Available from: http://www.lifetechnologies.com/in/en/home/life-science/pcr/real-time-pcr/qpcr-education/real-time-pcr-handbook.html?cid=rtpcrhandbook-rtpcr

25. Titiz A, Ozcakir O, Ceyhan S, Yilmaz YF, Unal A, Akyon Y. The presence of Helicobacter pylori in the larynx pathologies. *Auris Nasus Larynx.* 2008 Dec;35(4):534–8.

26. Ozyurt M, Gungor A, Ergunay K, Cekin E, Erkul E, Haznedaroglu T. Real-time PCR detection of Helicobacter pylori and virulence-associated cagA in nasal polyps and laryngeal disorders. *Otolaryngol--Head Neck Surg*. 2009 Jul;141(1):131–5.

27. Molina-Infante J, Gisbert JP. Optimizing clarithromycin-containing therapy for Helicobacter pylori in the era of antibiotic resistance. *World J Gastroenterol WJG*. 2014 Aug 14;20(30):10338–47.

28. Gisbert JP, Calvet X. Update on non-bismuth quadruple (concomitant) therapy for eradication of Helicobacter pylori. *Clin Exp Gastroenterol*. 2012;5:23–34.

29. Gleeson MJ, Clarke RC. Scott-Brown's Otorhinolaryngology: Head and Neck Surgery. 7 edition. London: CRC Press; 2008: 2130-39, 2192-204

30. Huang BY, Solle M, Weissler MC. Larynx: Anatomic Imaging for Diagnosis and Management. *Otolaryngol Clin North Am*. 2012 Dec;45(6):1325–61.

31. Flint PW, Haughey BH, Lund VJ, Niparko JK, Richardson MA. Cummings Otolaryngology - Head and Neck Surgery,5 edition. Philadelphia: Mosby; 2010. 894-901, 1482-88.

32. Armstrong WB, Netterville JL. Anatomy of the larynx, trachea, and bronchi. *Otolaryngol Clin North Am*. 1995 Aug;28(4):685–99.

33. Sataloff RT, Heman-Ackah YD, Hawkshaw MJ. Clinical Anatomy and Physiology of the Voice. *Otolaryngol Clin North Am.* 2007 Oct;40(5):909–29.

34. Noordzij JP, Ossoff RH. Anatomy and Physiology of the Larynx. *Otolaryngol Clin North Am*. 2006 Feb;39(1):1–10.

35. Robbins KT. Classification of neck dissection: current concepts and future considerations. *Otolaryngol Clin North Am.* 1998 Aug;31(4):639–55.

36. Robbins KT, Clayman G, Levine PA, Medina J, Sessions R, Shaha A, et al. Neck dissection classification update: revisions proposed by the American Head and Neck Society and the American Academy of Otolaryngology-Head and Neck Surgery. *Arch Otolaryngol Head Neck Surg*. 2002 Jul;128(7):751–8.

37. Robbins KT, Shaha AR, Medina JE, Califano JA, Wolf GT, Ferlito A, et al. Consensus statement on the classification and terminology of neck dissection. *Arch Otolaryngol Head Neck Surg.* 2008 May;134(5):536–8.

38. Altman KW. Vocal Fold Masses. *Otolaryngol Clin North Am.* 2007 Oct;40(5):1091–108.

39. Rosen CA, Gartner-Schmidt J, Hathaway B, Simpson CB, Postma GN, Courey M, et al. A nomenclature paradigm for benign midmembranous vocal fold lesions. *The Laryngoscope.* 2012 Jun;122(6):1335–41.

40. Singhal P, Bhandari A, Chouhan M, Sharma MP, Sharma S. Benign tumors of the larynx: a clinical study of 50 cases. *Indian J Otolaryngol Head Neck Surg* . 2009 Jan;61(Suppl 1):26–30.

41. Nunes RB, Behlau M, Nunes MB, Paulino JG. Clinical diagnosis and histological analysis of vocal nodules and polyps. *Braz J Otorhinolaryngol*. 2013 Aug;79(4):434–40.

42. Borkowski G, Sommer P, Stark T, Sudhoff H, Luckhaupt H. Recurrent respiratory papillomatosis associated with gastroesophageal reflux disease in children. *Eur Arch Oto-Rhino-Laryngol*. 1999;256(7):370–2.

43. Derkay CS, Wiatrak B. Recurrent respiratory papillomatosis: a review. *The Laryngoscope.* 2008 Jul;118(7):1236–47.

44. Goswami S, Patra TK. A Clinico-pathological study of Reinke'S oedema. *Indian J Otolaryngol Head Neck Surg.* 2003 Jul;55(3):160–5.

45. Bartlett RS, Heckman WW, Isenberg J, Thibeault SL, Dailey SH. Genetic characterization of vocal fold lesions: leukoplakia and carcinoma. *The Laryngoscope.* 2012 Feb;122(2):336–42.

46. Martins RHG, Dias NH, Santos DCD, Fabro AT, Braz JRC. Clinical, histological and electron microscopic aspects of vocal fold granulomas. *Braz J Otorhinolaryngol*. 2009 Feb;75(1):116–22.

47. Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg Oral Med Oral Pathol*. 1978 Oct;46(4):518–39.

48. Sadri M, McMahon J, Parker A. Management of laryngeal dysplasia: a review. *Eur Arch OtoRhinoLaryngol*. 2006 Sep;263(9):843–52.

49. Williamson JS, Biggs TC, Ingrams D. Laryngeal cancer: an overview. *Trends Urol Mens* Health. 2012 Nov 1;3(6):14–7.

50. Merletti F, Boffetta P, Ciccone G, Mashberg A, Terracini B. Role of tobacco and alcoholic beverages in the etiology of cancer of the oral cavity/oropharynx in Torino, Italy. *Cancer Res.* 1989 Sep 1;49(17):4919–24.

51. Markou K, Christoforidou A, Karasmanis I, Tsiropoulos G, Triaridis S, Constantinidis I, et al. Laryngeal cancer: epidemiological data from Northern Greece and review of the literature. *Hippokratia.* 2013 Oct;17(4):313–8.

52. Freije JE, Beatty TW, Campbell BH, Woodson BT, Schultz CJ, Toohill RJ. Carcinoma of the larynx in patients with gastroesophageal reflux. *Am J Otolaryngol*. 1996 Dec;17(6):386–90.

53. Francis DO, Maynard C, Weymuller EA, Reiber G, Merati AL, Yueh B. Reevaluation of gastroesophageal reflux disease as a risk factor for laryngeal cancer. *The Laryngoscope.* 2011 Jan;121(1):102–5.

54. Johnston N, Yan JC, Hoekzema CR, Samuels TL, Stoner GD, Blumin JH, et al. Pepsin promotes proliferation of laryngeal and pharyngeal epithelial cells. *The Laryngoscope.* 2012 Jun;122(6):1317–25.

55. Boffetta P, Richiardi L, Berrino F, Estève J, Pisani P, Crosignani P, et al. Occupation and larynx and hypopharynx cancer: an international case-control study in France, Italy, Spain, and Switzerland. *Cancer Causes Control*. 2003 Apr;14(3):203–12.

56. Agudelo D, Quer M, León X, Díez S, Burgués J. Laryngeal carcinoma in patients without a history of tobacco and alcohol use. *Head Neck.* 1997 May;19(3):200–4.

57. Genç R, Çağlı S, Yüce İ, Vural A, Okuducu H, Patıroğlu T, et al. The role of H. pylori in the development of laryngeal squamous cell carcinoma. *Dis Markers.* 2013;35(5):447–9.

58. Rezaii J, Tavakoli H, Esfandiari K, Ashegh H, Hasibi M, Ghanei G, et al. Association between Helicobacter pylori infection and laryngohypopharyngeal carcinoma: A case–control study and review of the literature. *Head Neck.* 2008 Dec 1;30(12):1624–7.

59. Califano J, Riet P van der, Westra W, Nawroz H, Clayman G, Piantadosi S, et al. Genetic Progression Model for Head and Neck Cancer: Implications for Field Cancerization. *Cancer Res.* 1996 Jun 1;56(11):2488–92.

60. Chu EA, Kim YJ. Laryngeal Cancer: Diagnosis and Preoperative Work-up. *Otolaryngol Clin North Am.* 2008 Aug;41(4):673–95.

61. Agrawal N, Ha PK. Management of early-stage laryngeal cancer. *Otolaryngol Clin North Am*. 2008 Aug;41(4):757–69, vi – vii.

62. Ulualp SO, Toohill RJ. Laryngopharyngeal reflux: state of the art diagnosis and treatment. *Otolaryngol Clin North Am.* 2000 Aug;33(4):785–802.

63. Patel D, Vaezi MF. Normal esophageal physiology and laryngopharyngeal reflux. *Otolaryngol Clin North Am*. 2013 Dec;46(6):1023–41.

64. Cunningham ET, Sawchenko PE. Central neural control of esophageal motility: a review. *Dysphagia*. 1990;5(1):35–51.

65. Bassotti G, Bacci G, Biagini D, David P, Alunni G, Pelli MA, et al. Manometric investigation of the entire esophagus in healthy subjects and patients with high-amplitude peristaltic contractions. *Dysphagia.* 1988;3(2):93–6.

66. Kahrilas PJ. GERD pathogenesis, pathophysiology, and clinical manifestations. *Cleve Clin J Med.* 2003 Nov;70 Suppl 5:S4–19.

67. Nwokediuko SC. Current trends in the management of gastroesophageal reflux disease: a review. *ISRN Gastroenterol.* 2012;2012:391631.

68. Cherry J, Margulies SI. Contact ulcer of the larynx. *The Laryngoscope*. 1968 Nov;78(11):1937–40.

69. Wright RA, Miller SA, Corsello BF. Acid-induced esophagobronchial-cardiac reflexes in humans. *Gastroenterology*. 1990 Jul;99(1):71–3.

70. Chung JH, Tae K, Lee YS, Jeong JH, Cho SH, Kim KR, et al. The significance of laryngopharyngeal reflux in benign vocal mucosal lesions. *Otolaryngol--Head Neck Surg.* 2009 Sep;141(3):369–73.

71. Beltsis A, Katsinelos P, Kountouras J, Kamarianis N, Zavos C, Pournaras A, et al. Double probe pH-monitoring findings in patients with benign lesions of the true vocal folds: comparison with typical GERD and the effect of smoking. *Eur Arch OtoRhinoLaryngol*. 2011 Aug;268(8):1169–74.

72. Belafsky PC, Postma GN, Koufman JA. The validity and reliability of the reflux finding score (RFS). *The Laryngoscope.* 2001 Aug;111(8):1313–7.

73. Belafsky PC, Postma GN, Amin MR, Koufman JA. Symptoms and findings of laryngopharyngeal reflux. *Ear Nose Throat J*. 2002 Sep;81(9 Suppl 2):10–3.

74. Cekin E, Ozyurt M, Erkul E, Ergunay K, Cincik H, Kapucu B, et al. The association between Helicobacter pylori and laryngopharyngeal reflux in laryngeal pathologies. *Ear Nose Throat J*. 2012 Mar;91(3):E6–9.

75. Snow JB, Wackym PA, Ballenger JJ. Ballenger's Otorhinolaryngology: Head and Neck Surgery. PMPH-USA; 2009. 1258 p.

76. Akbayir N, Başak T, Seven H, Sungun A, Erdem L. Investigation of Helicobacter pylori colonization in laryngeal neoplasia. *Eur Arch OtoRhinoLaryngol*. 2005 Mar;262(3):170–2.

77. Masoud N, Manouchehr K, Najmeh D, Monireh H. Lack of association between Helicobacter pylori and laryngeal carcinoma. *Asian Pac J Cancer Prev*. 2008 Mar;9(1):81–2.

78. Rubin JS, Benjamin E, Prior A, Lavy J, Ratcliffe P. The prevalence of Helicobacter pylori infection in benign laryngeal disorders. *J Voice*. 2002 Mar;16(1):87–91.

79. Borkowski G, Sudhoff H, Koslowski F, Hackstedt G, Radü HJ, Luckhaupt H. A possible role of Helicobacter pylori infection in the etiology of chronic laryngitis. *Eur Arch OtoRhinoLaryngol.* 1997;254(9-10):481–2.

80. Jaspersen D, Weber R, Diehl KL, Kind M, Arps H, Draf W. [Is chronic laryngitis associated with Helicobacter pylori? Results of a prospective study]. *Z Gastroenterol.* 1998 May;36(5):369–72.

81. Siupsinskiene N, Jurgutaviciute V, Katutiene I, Janciauskas D, Vaitkus S, Adamonis K. Helicobacter pylori infection in laryngeal diseases. *Eur Arch OtoRhinoLaryngol*. 2013 Aug;270(8):2283–8.

82. Espinoza MGC, Vazquez RG, Mendez IM, Vargas CR, Cerezo SG. Detection of the glmM gene in Helicobacter pylori isolates with a novel primer by PCR. *J Clin Microbiol.* 2011 Apr;49(4):1650–2.

83. Smith S-I, Oyedeji K-S, Arigbabu A-O, Cantet F, Megraud F, Ojo O-O, et al. Comparison of three PCR methods for detection of Helicobacter pylori DNA and

detection of cagA gene in gastric biopsy specimens. *World J. Gastroenterol*. 2004 Jul 1;10(13):1958–60.

84. Mégraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. *Clin Microbiol Rev.* 2007 Apr;20(2):280–322.

85. Aslan S, Yilmaz I, Bal N, Sener M, Butros R, Demirhan B, et al. Investigation of Helicobacter pylori in tonsillary tissue with Pronto Dry test and pathologic examination. *Auris Nasus Larynx.* 2007 Sep;34(3):339–42.

86. Banjara H, Mungutwar V, Singh D, Gupta A, Singh S. Demographic and videostroboscopic assessment of vocal pathologies. *Indian J Otolaryngol Head Neck Surg*. 2012 Jun;64(2):150–7.

87. Licitra L, Bernier J, Grandi C, Locati L, Merlano M, Gatta G, et al. Cancer of the larynx. *Crit Rev Oncol Hematol*. 2003 Jul;47(1):65–80.

88. Maasland DHE, van den Brandt PA, Kremer B, Goldbohm RAS, Schouten LJ. Alcohol consumption, cigarette smoking and the risk of subtypes of head-neck cancer: results from the Netherlands Cohort Study. *BMC Cancer.* 2014;14:187.

89. Menach OP, Patel A, Oburra HO. Demography and histologic pattern of laryngeal squamous cell carcinoma in kenya. *Int J Otolaryngol.* 2014;2014:507189.

90. Sulica L. Laryngoscopy, Stroboscopy and Other Tools for the Evaluation of Voice Disorders. *Otolaryngol Clin North Am*. 2013 Feb;46(1):21–30.

91. Lipan MJ, Reidenberg JS, Laitman JT. Anatomy of reflux: a growing health problem affecting structures of the head and neck. *Anat Rec B New Anat.* 2006 Nov;289(6):261–70.

92. Koufman JA, Amin MR, Panetti M. Prevalence of reflux in 113 consecutive patients with laryngeal and voice disorders. *Otolaryngol Head Neck Surg*. 2000 Oct;123(4):385–8.

93. Copper MP, Smit CF, Stanojcic LD, Devriese PP, Schouwenburg PF, Mathus-Vliegen LM. High incidence of laryngopharyngeal reflux in patients with head and neck cancer. *The Laryngoscope.* 2000 Jun;110(6):1007–11.

PATIENT INFORMATION SHEET

You are requested to participate in this study which aims at finding the presence of a bacteria named *Helicobacter pylori* in voice box diseases. In this study you will be asked details of your disease in the form of a questionnaire. An endoscopic visualization of your voice box will be done to assess your disease and also if there is an acidity problem in your voice box. After obtaining a written consent ,you will undergo a microscope assisted visualization of voice box under anaesthesia and tissue will be taken for biopsy. A part of the tissue will be taken for the study and sent for a test called PCR analysis which detects the bacteria. There would not be any risk to you by participating in this study. You need not pay any extra charges for the test. You can withdraw from the study at any moment you feel so and this in no way will compromise your treatment. Your participation in the study will remain confidential and shall be known only to investigators.

INFORMED VALID CONSENT

Study number-

Hospital number-

Participant's name-

Date of birth/age

I, _____son/daughter/wife of _____have been explained in my own language about the proposed study. It has been explained that this study involves taking tissue from the lesion on my voice box for testing the presence of a bacteria (H. pylori) during microlaryngoscopic biopsy under GA . This is an operation routinely done for patients diagnosed with my disease (clinically diagnosed laryngeal cancers/ vocal cord polyp/ papilloma / cyst/ nodule).It has been explained to me that there is no additional risk in the study and that I am free to withdraw from the study any time I want and it will not in any way compromise the treatment, the ENT doctors are giving me. I understand that my identity and participation will not be revealed in any information released to third parties. I am giving this consent on my own free will. I hereby give my full valid consent for participating in the proposed study.

Name of patient

Signature

Date

Name of Doctor

Signature

Date

Name of witness

Signature.

Date

தகவல் தாள்

ஹெலிகோபாக்டர் பைலோரை என்னும் கிருமி குரல்வளை நோய்களில் எவ்வளவு பரவலாக உள்ளது பற்றிய இந்த ஆய்வில் கலந்துகொள்ள கேட்டுக்கொள்ளப்படுகிறீர்கள். உங்கள் நோய்யைப் பற்றிய கேள்விகள் சில கேட்கப்படும். உங்கள் குரல்வளையை பார்க்க என்டோஸ்கோப் என்னும் ஒரு கருவி பயன்படுத்தப்படும், நீங்கள் சம்மதித்தபிறகு உங்கள் குரல்வளை தசை மைக்ரோஸ்கோப் மூலம் பரிசோதனைக்கு, மயக்க மருந்து கொடுத்தபின் எடுக்கப்படும். இந்த தசையின் ஒரு பகுதி பி.சி.ஆர். என்னும் பரிசோதனைக்கு அனுப்பப்படும். கூடுதல் ஆபத்து இதனால் கிடையாது. கூடுதல் செவும் இதனால் கிடையாது. இந்த ஆய்விலிருந்து எப்பொழுது வேண்டும் என்றாலும் விலகிக்கொள்ளலாம். அதனால் உங்கள் சிகிச்சைக்கு எந்த இடையூறும் வராது. உங்கள் அடையாளம், ஆய்வு செய்பவரை தவிர வேறு எவருக்கும் தெரிவிக்கப்படாது.

சம்மத தாள்

வரிசை எண் :

பெயர் :

ஆஸ்பத்திரி எண் :

வயது :

..... ஆகிய எனக்கு இந்த ஆய்வைக் குறித்து என்னுடைய மொழியில் விளக்கப்பட்டுள்ளது. என் குரல்வளையிலிருந்து மயக்க மருந்து கொடுத்து, மைக்ரோஸ்கோப் மூலம் தசை எடுக்கப்படும். அது ஹெலிகோபாக்டர் பைலோரை என்னும் கிருமிக்காக பரிசோதிக்கப்படும் இதனால் கூடுதல் ஆபத்து எதுவும் இல்லை என்றும், எப்பொழுது வேண்டும் என்றாலும் இந்த ஆய்விலிருந்து விலகிக்கொள்ளலாம் என்றும் விளக்கப்பட்டுள்ளது. என் அடையாளம் ஆய்வு செய்பவரை தவிர வேறு எவருக்கும் தெரிவிக்கப்படாது என்றும் அறிவிக்கப்பட்டுள்ளது. இந்த ஆய்வில் கலந்துக்கொள்ள என் முழு சம்மதத்தை அளிக்கிறேன்.

பெயர்:

பங்குபெறுவோரின் கையொப்பம் :

சாட்சியின் பெயர் : டாக்டர் பெயர்:

சாட்சியின் கையொப்பம் : டாக்டர் கையொப்பம்:

தேதி:

रोगी सूचना पत्र

आपको इस अध्ययन में शामिल होने की अनुरोध कर रहे हैं। यह अध्ययन ब्लड उत्पादन करने वाले ऊतक के रोगों में हीलिकोबैक्टर पाइलोरी नामक एक किटाणु की उपस्थिति देखने के लिए किया जा रहा है। इसमें आपसे आपकी बिमारी के बारे में कुछ प्रश्न किया जायेंगे। इंडोस्कोपिक दृश्य से आपकी बीमारी की गंभीरता का आकलन किया जाएगा। लिखित सहमति प्राप्त करने के बाद माइक्रोस्कोप की मदद से बायोप्सी ली जाएगी। बायोप्सी का एक हिस्सा पी सी आर नामक एक विश्लेषण परीक्षण के लिए भेजा जाएगा। इस परीक्षण से इस किटाणु का उपस्थिति मालूम होता है। इस अध्ययन में कोई विशेष खतरा नहीं होगा और आपको कोई अतिरिक्त पैसा नहीं देना होगा। आप किसी भी क्षण इस अध्ययन से अपना नाम वापस ले सकते हैं और इससे आपके इलाज में कोई समझौता नहीं होगा। अध्ययन में आपकी भागीदारी केवल शोधकर्ताओं को पता रहेगी और अन्य लोगों से गोपनीय रहेगी।

अवगत वैध सहमति

नंबर :
अस्पताल नंबर :
प्रतिभागी का नाम :
उम्र :

मुझे प्रस्तावित अध्ययन के बारे में अपनी समझ की भाषा में समझाया गया है। मुझे बताया गया है कि मेरे ध्वनि उत्पादन करने वाले डब्बे में बीमारी है और इसके इलाज के लिए वहाँ से एक टुकड़ा नाच के लिए लेने की जरूरत है। इसके साथ ही एक टुकड़ा इस अध्ययन के लिए भी लिया जायेगा जिसमें हेलिकोबैक्टर पाइलोरी नामक किटाणु का परीक्षण किया जायेगा। यह ओपरेशन नियमित तौर पर मेरे जैसे बिमारी वाले मरीजों पर किया जाता है। इस अध्ययन में शामिल होने से मुझे कोई विशेष खतरा नहीं होगा और मैं किसी भी समय इस अध्ययन से अपना नाम वापस ले सकता हूँ। इससे मेरे इलाज में कोई समझोता नहीं होगा। मेरी भागीदारी और पहचान गोपनीय रखी जायेगी। मैं अपनी सहमति से इस अध्ययन में शामिल हो रहा हूँ। मुझे यह सब अपनी भाषा में समझा दिया गया है और मैं इस अध्ययन में भाग लेने के लिए अपनी पूरी सहमति देता हूँ।

प्रतिभागी का नाम :
हस्ताक्षर :
तिथी :

डाक्टर का नाम :
हस्ताक्षर

रिश्तेदार का नाम :
हस्ताक्षर :
तिथी :

CLINICAL RESEARCH FORM

Serial no:

Hospital no:

Name of patient:

Age: Sex:

Address:

Occupation:

Level of voice use:

Telephone number:

Email:

Presenting Complaint

	Yes/ No	Duration
Hoarseness		
Throat pain		
Difficulty in swallowing (Solids/ liquids/ pills)		
Foreign body sensation throat		
Cough		
Difficulty in breathing		
History of voice abuse		
History of belching/ retrosternal burning		
Throat clearing		
Neck swelling		

Past History

	Yes/ No
Diabetes	
Hypertension	
Tuberculosis	
Bronchial asthma	
Others	

Drug History

	Yes/ No
Proton pump inhibitors (within 4 weeks)	
H2 antagonists (within 4 weeks)	
Antibiotics (within 4 weeks)	
Others	

Personal History:

	Yes/ No	Duration
Smoker		
Tobacco chewer		
Alcoholic		
Diet		
Dinner time		
Bed time		

Examination Findings

Indirect Laryngoscopy findings:

GRBAS

Pitch: Increased /decreased/ diplophonia

Reflux Symptom Index Questionnaire 0-no problem

5-severe problem

	Symptoms	0	1	2	3	4	5
1	Hoarseness or problem with voice						
2	Clearing your throat						
3	Throat mucus or post nasal drip						
4	Difficulty in swallowing solids, liquids or pills						
5	Coughing after you have eaten or after lying down						
6	Breathing difficulties or choking episodes						
7	Troublesome or annoying cough						
8	Sensation or lump in your throat						
9	Heartburn or stomach acid coming up						

Total Score=

Endoscopic Findings:

Diagnosis-

Reflux Finding Score

1) Subglottic oedema

absent	0
present	2

2) Ventricular obliteration

partial	2
complete	4

3) Vocal fold oedema

mild	1
moderate	2
severe	3
polypoidal	4

4) Posterior commissure hypertrophy

mild	1
moderate	2
severe	3
obstructing	4

5) Granuloma/ Granulation tissue

absent	0
present	2

6) Erythema/ Hyperaemia

arytenoids only	2
diffuse	4

7) Diffuse laryngeal oedema

mild	1
moderate	2
severe	3
obstructing	4

8) Thick endolaryngeal mucus

absent	0
present	2

Total Score =

DOA: DOS: DOD:

Operative findings:

Biopsy report:

Giemsa report:

PCR report:

EXCEL DATA SHEET

ser	hosp no	age	Sex	occupatio	level	Hoa	duration	belc	durati	Throa	durat	forei	duratic	swallo	durati	breat	durat	voice	duratic	cough	durati	throat	dura	neck	durat	smok	dural	tobac	duratic
1	728575f	60	1	subinspec	3	1	2 years	2		2		1	3 years	2		2		1	30 year	2		1	3 yea	2		1	4 yea	1	34 year
2	747426F	62	1	forestry d	3	1	1 1/2 years	1	2mon	1	13 mc	2		1	2mon	2		2		2		1	1 mc	2		1	20 ye	2	
3	709641f	34	1	engineer	4	1	1 1/2 years	1	4 year	2		2		2		2		1	2 years	2		1	4 yea	2		1	15 ye	2	
4	749680f	28	2	housewife	4	1	3 months	1	6 year	1	3 mor	1	3 mont	1	3 mor	1	3 mo	2		1	7 year	1	7 yea	2		2		2	
5	385653f	43	1	police	2	1	10 years	1	6 year	2		2		2		2		1	15 year	2		1	10 ye	2		1	5 yea	2	
6	653164f	63	1	Manual la	4	1	1 year	2		2		1	1 mont	2		2		2		1	1 mon	1	1 mc	2		1	35 ye	2	
7	280607d	77	1	Manual la	4	1	3 months	2		2		2		2		2		2		2		2		2		1	40 ye	2	
8	736370c	47	1	Professor	2	1	5 years	2		2		1	3 years	2		2		1	5 years	2		1	10 ye	2		1	25 ye	2	
9	683367f	67	1	Farmer	2	1	3 months	2		2		2		2		2		2		1	2 mon	1	3 mc	2		1	35 ye	2	
10	743431f	46	1	Carpenter	2	1	1 year	1	7 year	2		1	2 years	2		2		1	10 year	2		1	2 yea	2		2		2	
11	776178f	49	1	Manual la	3	1	4 years	2		2		2		2		2		1	15 year	2		2		2		1	15 ye	1	1 1/2 ye
12	066870f	75	1	Driver	4	1	2 month	2		2		2		2		1	2 yea	2		1	2 year	2		2		1	35 ye	2	
13	760049f	44	1	Shop keep	3	1	2 years	2		2		2		2		2		1	10 year	2		1	15 ye	2		2		2	
14	183647f	48	1	Architect	4	1	1 month	2		2		2		2		2		2		2		2		2		1	30 ye	2	
15	798019f	33	1	Manual la	3	1	1 month	2		2		2		2		2		2		2		2		2		1	15 ye	2	
16	799999f	41	1	Servicema	4	1	8 months	2		2		2		2		2		1	10 year	2		1	5 yea	2		2		1	15 year
17	799819f	59	1	Govt emp	2	1	6 months	2		2		2		2		2		1	10 year	2		2		2		1	42 ye	2	
18	805861f	52	1	Lawyer	2	1	2 1/2 month	1	2 year	2		2		2		2		1	30 year	2		1	2 mc	2		2		1	3 years
19	749337f	60	2	Housewife	4	1	11 months	2		2		2		2		2		2		2		2		2		2		2	
20	779521f	35	1	Student	3	1	1 year	1	7 mor	2		1	7 mont	2		2		2		2		2		2		2		2	
21	773541f	28	1	Manual la	2	1	2 months	2		2		1	2 mont	2		2		1	2 mont	2		1	2 mc	2		1	2 yea	2	
22	796345F	56	1	Printing w	4	1	2 years	2		2		2		2		2		2		2		2		2		2		2	
23	812613f	56	1	Manual la	3	1	7 months	2		2		2		2		2		1	30 year	2		1	7 yea	2		1	26 ye	1	3 years
24	769023f	44	1	Teacher	2	1	2 months	2		2		2		2		2		1	18 year	2		1	10 ye	2		1	22 ye	2	
25	809798f	44	2	Nurse	3	1	2 years	1	5 year	2		2		2		2		2		2		2		2		2		2	
26	817582F	50	1	engineer	2	2		1	3 year	2		1	2 1/2 y	2		2		1	2 years	2		2		2		2		2	
27	811353f	31	1	Business r	3	1	4 months	2		2		2		2		2		1	3 years	2		1	4 mc	2		2		2	
28	429092f	54	1	Labourer	4	1	3 months	2		2		2		2		2		2		2		2		2		1	25 ye	2	
29	694622f	45	1	Business r	3	1	2 years	1	12 yea	2		2		2		2		2		2		1	2 yea	2		1	4 yea	1	20 year
30	808335	69	2	Housewife	4	1	2 years	2		2		2		2		2		2		2		2		2		2		2	
31	268290f	20	2	Housewife	4	1	2 years	2		2		2		2		2		2		2		2		2		2		2	
32	694753f	43	1	Porter	2	1	20 years	1	15 yea	2		1	10 yea	2		2		1	20 year	2		1	10 ye	2		2		1	20 year
33	759528f	47	1	Business r	3	1	7 months	2		2		1	3 mont	2		1	3 mo	2		1	3 mon	1	3 mc	2		1	30 ye	1	30 year
34	814149f	40	1	Farmer	3	1	2 months	2		1	2 mor	1	1 mont	2		2		2		2		2		2		2		1	6 years
35	814541f	56	1	Operator	2	1	2 years	2		2		2		2		2		1	18 year	2		2		2		2		1	2 1/2 ye
36	821627f	37	1	Servicema	3	2		1	7 year	2		1	10 yea	2		2		1	6 mont	2		2		2		1	2 yea	2	
37	820175f	44	1	Farmer	3	1	2 months	1	10 yea	2		1	2 years	2		2		1	2 years	2		1	5 mc	2		1	25 ye	1	20 year
38	026218f	76	1	Manual la	3	1	25 days	2		2		2		2		2		2		2		1	10 ye	2		1	20 ye	2	
39	723713f	52	1	Constructi	2	1	7 months	1	10 yea	2		1	3 mont	2		2		1	30 year	2		1	7 mc	2		1	10 ye	2	
40	766527d	70	1	Teacher	2	1	6 months	2		2		2		2		2		1	30 year	2		2		2		1	55 ye	2	
41	778435d	49	1	engineer	3	1	3 months	1	3 year	2		2		2		2		1	20 year	2		1	2 yea	2		1	20 ye	2	
42	833322f	41	1	Contracto	2	1	3 months	2		2		2		2		2		1	8 years	2		1	1 mc	2		1	3 yea	2	
43	829140f	39	1	Business r	3	1	4 months	1	4 mor	2		2		2		2		2		2		2		2		2		1	1/week
44	829877f	33	1	Business r	3	1	5 months	2		2		2		2		2		2		2		2		2		2		2	
45	146671d	37	1	constructi	2	1	4 months	1	20 yea	2		2		2		2		1	10 year	2		2		2		2		2	
46	211200c	71	1	Manual la	3	1	12 years	2		2		2		2		2		2		2		2		2		2		2	
47	836034f	59	1	Carpenter	3	1	10 years	2		2		2		2		2		1	40 year	2		1	2 yea	2		1	34 ye	1	
48	829848f	50	1	LIC Agent	3	1	1 1/2 years	2		2		1	6 mont	2		2		2		2		1	6 mc	2		2		2	
49	368891D	46	1	Policemar	2	1	2 months	2		2		2		2		2		1	23 year	2		1	1 yea	2		2		2	
50	833912f	64	1	Manual la	3	1	8 months	2		2		1	1 week	2		2		2		2		1	10 ye	2		1	35 ye	2	

alcoho	durat	DM :	dura	HTN :	durat	TB :	dur	PPI :	Antib	dinner	bed ti	GRBAS	RSI	RFS	FINDINGS	Diagnosis	Biopsy	Giemsa 1-	PCR 1- po
1	stopp	2		1	4 yea	2		2	2	9.00 pn	10.30	G1RIB2	10	4	Proliferati	carcinoma	moderate	2	2
2		2		2		2		2	2	9.00 pn	10.30	GIRIBI	10	6	Proliferati	transglott	moderate	2	2
1	15 ye	2		2		2		2	2	10.30 p	11.00	G2R2B0	12	11	Large broa	vocal cord	benign vo	2	2
2		2		2		2		2	2	8.00 pn	9.00 p	G1RIB1	20	11	Huge ped	right voca	ulceratior	2	2
1	5 yea	2		2		2		2	2	no fixe	imme	G2R2BO	9	8	Haemorrh	left vocal	benign vo	2	2
1	15 ye	2		2		2		2	2	11.00 p	imme	G1R1B1	8	2	Papilloma	papilloma	florid pap	2	2
2		2		1	5 yea	2		2	2	8.00 pn	9.00 p	G3R1B0	5	6	Exophytic	carcinoma	moderate	2	2
1	5 yea	2		2		2		2	2	8.00 pn	10.30	G2R2B0	12	7	Polyp at t	left vocal	benign vo	2	2
2		2		1	1 mor	2		2	2	8.00 pn	8.30 p	G3R2B0	6	5	Haemorrh	right voca	benign vo	2	2
2		2		2		2		2	2	11.00 p	11.30	G3R2B0	11	5	Polyp in tl	right voca	benign vo	2	2
2		1	5 yea	2		2		2	2	10.30 p	12.00	G2R1B1	3	3	Sessile ha	right voca	benign vo	2	2
2		2		2		2		2	2	10.00 p	10.30	G3R2B1	6	6	Irregular k	malignanc	moderate	2	2
2		2		2		2		2	2	10.30 p	11.00	G2R2B0	6	1	Pedunculá	left vocal	benign vo	2	2
1	2 yea	2		2		2		2	2	8.30 pn	1.30a	G2R1B0	3	4	Whitish pa	chronic la	subepithe	2	2
1	5 yea	1	1 yea	2		2		2	2	9.00 pn	11.00	G3R3B0	6	9	Small whi	Vocal corc	Epithelial	2	2
2		2		2		2		2	2	9.30 pn	11.30	G3R3B1	5	3	Polyp at t	left vocal	benign vo	2	2
2		2		2		2		2	2	11.00pr	2.30 a	G2R2B0	4	3	keratotic p	right voca	epithelial	2	2
2		2		2		2		2	2	9.30 pn	10.30	G3R3B0	11	6	Pedunculá	left vocal	left vocal	2	2
2		1	6 yea	2		2		2	2	11.00pr	11.30	G2R1B0	5	3	Pale polyp	right voca	benign vo	2	2
2		2		2		2		2	2	9.00 pn	11.00	G3R2S1	8	3	Polyp at t	right voca	benign vo	2	2
1	2 yea	2		2		2		2	2	8.00 pn	11.00	G2R2B0	8	3	Polyp at t	right voca	benign vo	2	2
2		2		1	1 mor	2		2	2	8.00 pn	11.30	G3R3B1	8	6	Slough co	malignanc	well diffe	2	2
1	3 yea	1	8 yea	1	7 yeai	2		2	2	12.00ar	12.30	G3R3B2	8	3	Proliferati	carcinoma	well to mo	2	2
2		2		1	10 yea	2		2	2	11.00pr	11.30	G2R2B0	5	7	Broad bas	left vocal	benign fib	2	2
2		2		2		2		2	2	10.30 p	11.00	G3R3B1	7	6	polyp at tl	right voca	benign vo	2	2
2		2		2		2		2	2	10.00 p	10.10	G0R0B0	7	6	Small sess	left vocal	benign vo	2	2
2		2		2		2		2	2	9.30 pn	11.00	G3R3B0	7	1	Haemorrh	right voca	benign vo	2	2
2		2		1	5 yea	2		2	2	10.00pr	10.30	G3R3B2	7	0	Exophytic	malignanc	well to mo	2	2
2		1	2 yea	2		2		2	2	9.30 pn	11.30	G3R3B0	10	7	polyp at tl	right voca	benign vo	2	2
2		2		2		2		2	2	8.30 pn	10.00	G2R2B1	6	5	Papilloma	vocal cord	Squamous	2	2
2		2		2		2		2	2	8.30pm	10.00	G3R2B2	6	13	Irregular g	granuloma	epithelial	2	2
2		2		1	3 yea	2		2	2	11.00 p	11.30	G2R2B1	9	10	Pedunculá	right voca	benign vo	2	2
1	30 ye	2		2		2		2	2	9.00 pn	11.00	G2R2B1	12	0	Proliferati	Carcinoma	well to mo	2	2
2		2		2		2		2	2	10.30 p	11.00	G3R3B0	9	5	Polyp in tl	left vocal	benign vo	2	2
1	40 ye	1	6 yea	1	15 yea	2		2	2	9.00 pn	9.30 p	G3R3B1	8	6	Papilloma	papilloma	Benign pa	2	2
2		2		2		2		2	2	10.30 p	12.00	G1R1B0	8	8	Reddish g	right vent	benign ha	2	2
1	15 ye	2		2		2		2	2	9.00 pn	10.00	G3R2B0	16	5	Small poly	left vocal	benign vc	2	2
2		2		1	10 yea	2		2	2	8.00pm	10.00	G2R1B2	8	7	Exophytic	carcinoma	well to mo	2	2
2		2		2		2		2	2	10.30pr	11.00	G3R2B0	12	9	Small hae	right voca	benign vo	2	2
1	50 ye	1	1 we	1	1 wee	2		2	2	8.00pm	9.00p	G3R2B3	7	10	Proliferati	malignanc	moderate	2	2
2		2		2		2		2	2	10.00 p	11.30	G3R2B0	15	9	Multilobu	left vocal	benign po	2	2
1	10 ye	2		2		2		2	2	10.00pr	11.00	G3R3B0	9	5	Proliferati	malignanc	well diffe	2	2
2		2		2		2		2	2	12.30ar	1.00 a	G3R3B0	8	6	Haemorrh	left vocal	left vocal	2	2
2		2		2		2		2	2	8.00pm	8.30p	G2R2B1	6	5	Haemorrh	right voca	benign vo	2	2
2		2		2		2		2	2	7.00pm	9.30p	G2R2B0	8	8	Polyp in tl	left vocal	benign vo	2	2
2		2		2		2		2	2	8.30 pn	10.00	G3R3B3	7	5	Multiple v	recurrent	laryngeal	2	2
2		2		2		2		2	2	10.00pr	11.30	G3R3B0	9	4	Haemorrh	left vocal	benign lef	2	2
2		1	10 ye	2		2		2	2	9.30 pn	11.00	G2R2BO	8	10	Ulceropro	Carcinoma	focal seve	2	2
2		2		2		2		2	2	10.00 p	1.00 a	G2R2B0	7	4	Whitish ke	Vocal corc	epithelial	2	2
2		2		2		2		2	2	10.00pr	12.00	G3R1B3	11	3	Ulceropro	malignanc	well to mo	2	2

51	835998f	29	1	Farmer	3	1	4 years	2		2		1	3 years	2		2		2		2		2		2		1	10/da	1	4 times
52	824796f	53	1	Private co	4	1	6months	2		2		2		2		2		2		2		2		2		2		2	
53	831693f	36	2	Housewif	4	1	2 years	2		2		1	2 years	2		2		2		1	2 yea	2		2		2	
54	838135f	40	1	Tea Seller	3	1	3months	2		2		2		2		2		1	15year	2		1	5 mo	2		1	5yeai	1	10 year
55	821761f	36	1	LIC Agent	3	1	7 years	1	5years	2		2		2		2		1	10 year	2		1	6 yea	2		1	16 ye	2	
56	836696f	27	1	Business	3	1	6months	2		2		2		2		2		2		2		2		2		2	
57	829439f	74	1	Farmer	4	1	4 years	2		1	2 mor	1	6 mon	2		2		2		2		2		1	5 yea	1	15 year
58	825798f	58	1	Manual la	4	1	8 months	2		2		2		2		2		2		1	8 mo	2		1	40 ye	1	20 year
59	834860f	35	1	Farmer	4	1	3 months	2		2		2		2		2		2		1	3 mo	2		1	10 ye	2	
60	680381f	45	2	Housewif	4	1	3 years	1	3 year	2		2		2		2		1	10 year	2		2		2		2	
61	018056c	29	1	Medical re	2	1	7 years	2		1	1 1/2	2		2		2		1	7 years	2		1	5 yea	2		2		2	
62	815049F	51	1	Shop keep	4	1	1 year	2		2		2		2		2		1	10 year	2		2		2		1	25 ye	2	
63	840880f	65	1	Farmer	4	1	2 1/2 years	2		2		2		2		1	2 yea	2		2		2		2		1	50 ye	2	
64	838576f	34	1	Teacher	2	1	3months	2		2		2		2		2		1	6 years	2		1	3 mo	2		2		2	
65	848778f	43	1	Businessn	3	1	3 years	2		2		2		2		2		2		2		1	3 yea	2		2		2	
66	849456f	40	1	Private co	3	1	15 years	2		2		1	9 years	2		2		1	10 year	2		1	4 yea	2		1	27 ye	1	20 year
67	860006f	27	2	Housewif	4	1	5years	2		2		2		2		1	6 mor	2		2		2		2		2	
68	858811f	57	1	Businessn	3	1	2 years	2		2		2		2		2		1	14 year	1	1 mon	1	1 mo	2		1	10 ye	2	
69	862617f	57	1	Warden	3	1	2 years	2		1	2 mor	2		1	1 mon	2		1	28 year	1	1 mon	1	2 mo	2		1	20 ye	2	
70	857037f	48	1	Business r	3	1	2 years	2		2		2		2		2		1	10 year	2		2		2		1	5 yea	2	
71	862376f	50	1	Carpenter	3	1	9 months	2		2		2		2		2		2		2		2		2		1	20 ye	2	
72	867230f	48	1	Farmer	4	1	4 years	2		2		1	3 mon	2		2		2		2		1	4 mo	2		1	12 ye	2	
73	617616f	53	1	engineer	3	1	1 year	2		2		2		2		2		1	20 year	2		2		2		2		2	
74	809516f	57	1	Housewif	4	1	25 years	1	3 year	2		1	25 yea	2		1	10 ye	2		2		1	3 yea	2		2		2	
75	871662f	65	1	Retired le	2	1	4 months	2		2		2		2		2		1	30 year	2		2		2		2		2	
76	860621f	65	1	Pastor	2	1	6 months	2		1	1 mor	2		2		2		1	40 year	2		2		2		1	10 ye	2	
77	876935f	60	1	Tea shopk	4	1	2 years	2		2		1	2 mon	2		1	4 mor	1	2 mont	1	7 mon	1	4 mo	2		2		1	10 year
78	870933f	25	1	engineer	3	1	3 months	2		2		1	2 mon	2		2		1	5 years	2		1	2 mo	2		1	3 yea	1	3 years
79	882577f	44	1	Fish seller	3	1	3 months	2		2		2		2		2		1	30 year	2		1	3 mo	2		1	15 ye	1	15 year
80	844618f	45	1	Business	4	2		2		1	2 mor	1	2 mon	1	1 mor	1	1 wee	2		1	1 mon	1	2 mo	2		1	20 ye	2	
81	879915f	39	1	Vegetable	3	1	6 months	1	5 year	2		2		2		2		1	15 year	2		1	6 mo	2		2		2	
82	878057f	61	1	Teacher	2	1	2 months	2		2		2		2		2		1	40 year	2		2		2		1	40 ye	1	40 year
83	866104d	48	2	Drama act	2	1	1 year	1	2 year	2		2		2		1	8 mor	1	4 years	2		1	2 yea	2		2		2	
84	882615f	76	1	Shop keep	4	1	6months	2		2		1	2 1/2 n	2		1	4 mor	2		2		1	10 ye	2		1	40 ye	2	
85	881624f	35	1	Driver	4	1	7 years	2		2		2		2		2		2		2		2		2		1	35 ye	2	
86	886394f	40	2	Contracto	2	1	4 months	2		2		2		2		2		1	10 year	2		2		2		2		1	5 years
87	380069c	56	1	Postman	2	1	4 years	1	5 year	2		2		2		2		1	6 years	2		1	5 yea	2		1	5 yea	2	
88	886917f	74	1	Waiter	3	1	3 months	2		2		1	1 mon	2		2		1	25 year	1	5 year	2		2		1	50 ye	2	
89	891027f	36	1	Civil Engir	3	1	3 months	1	10 yea	2		1	3 mon	2		2		1	10 year	1	3 mon	1	3 mo	2		1	`18 ye	1	20 year
90	666518d	35	1	Servicema	3	1	2 months	2		2		1	2 mon	2		2		1	15 year	2		1	2 mo	2		2		2	
91	891094f	47	1	Teacher	2	1	2 months	1	3 year	2		2		2		2		1	24 year	1	10 yea	1	10 ye	2		1	20 ye	occasional	
92	884718f	29	1	Works in F	3	1	4 months	1	6 year	2		1	4 mon	2		2		1	10 year	2		1	4 mo	2		1	3 yea	2	
93	893999f	29	1	Teacher	2	1	1 month	2		2		2		2		2		1	5 years	2		1	2 mo	2		2		2	
94	391234f	43	1	Actor	2	1	1 year	2		2		2		2		1	3 mor	1	10 year	2		2		2		1	20 ye	1	20 year
95	897419f	50	2	Housewif	4	1	2 months	2		2		2		1	6 mon	2		2		2		2		2		2	
96	819670F	31	1	engineer	3	1	8 months	1	3 year	2		1	8 mon	2		2		1	3 years	1	1 year	1	8 mo	2		1	3 yea	2	
97	865371f	36	1	Farmer	3	1	6months	1	6 year	2		2		2		2		1	10 year	1	4 year	1	6 mo	2		1	10 ye	2	
98	767830f	36	1	Shop keep	3	1	4 years	1	10 yea	2		1	4 years	2		2		1	12 year	2		1	12 ye	2		1	15 ye	2	
99	813304d	49	1	Driver	3	1	3 months	2		2		1		2		2		1	10 year	2		1	1o ye	2		2		2	
##	005964g	52	1	Servicema	4	1	3 months	2		2		1	3 mon	2		2		1	25 year	2		1	3 mo	2		2		2	

2		2		2		2	2	2	9.00pm	10.00	G2R2B0	11	6	Whitish p	left vocal	benign po	2	2
1	10 ye	2		1	2 yea	2	2	2	10.00 p	11.00	G2R1B0	6	6	Haemorrh	left vocal	Left vocal	2	2
2		2		2		2	2	2	8.30pm	9.00p	G3R3B0	11	6	Large ped	left vocalc	benign vo	2	2
1	occas	2		2		2	2	2	8.30pm	10.30	G2R2B1	8	6	Haemorrh	left vocal	benign vo	2	2
2		1	2yea	2		2	2	2	10.30pr	11.00	G2R2B0	14	5	Broad bas	Right voca	vocal cord	2	2
2		2		2		2	2	2	9.30pm	11.00	G2R1B0	6	3	Polypoidal lesion involving the			2	2
2		2		2		2	2	2	9.30pm	10.00	G2R2B1	10	3	Exophytic	transglott	well to mo	2	2
1	15ye	1	1mo	2		2	2	2	8.30pm	10.00	G3R2B2	8	6	Verrucous lesion see		Florid ept	2	2
1	10 ye	2		2		2	2	2	9.30pm	10.30	G2R2B0	6	7	Broad bas	left vocal	benign vo	2	2
2		2		1	3 yea	2	2	2	10.30pr	11.30	G2R2B1	8	3	Sessile po	right voca	benign po	2	2
2		2		2		2	2	2	8.00pm	11.30	G3R2B1	9	8	Pedunculá	Right voca	benign vo	2	2
1	10 ye	1	4 yea	1	4 yea	2	2	2	8.00pm	9.00p	G3R2B1	6	3	Broad bas	right voca	vocal cord	2	2
2		2		2		2	2	2	9.00pm	10.00	G3R1B3	7	1	Proliferati	carcinoma	carcinoma	2	2
2		2		2		2	2	2	9.00 pn	10.00	G2R1B0	7	6	Haemorrh	right voca	benign vo	2	2
2		2		2		2	2	2	12.00ar	1.00a	G2R2B0	8	3	Broad bas	left vocal	benign vo	2	2
2		2		2		2	2	2	11.00pr	12.00	G2R1B1	12	6	Large poly	right voca	Benign vo	2	2
2		2		2		2	2	2	11.00pr	12.00	G3R1B3	6	4	Multiple p	Laryngeal	Laryngeal	2	2
2		2		1	2 yea	2	2	2	10.00pr	11.30	G2R2B0	11	6	Mucosa co	Carcinoma	moderate	2	2
2		2		2		2	2	2	9.00pm	10.00	G3R2B1	15	8	orm sinus	carcinoma	Suggestive	2	2
2		2		2		2	2	2	10.00pr	11.00	G2R1B0	5	5	Polyp see	Left vocal	Benign vo	2	2
2		2		2		2	2	2	10.30pr	11.30	G3R2B0	6	7	Ulceropro	carcinoma	moderate	2	2
2		2		2		2	2	2	11.00pr	11.20	G3R3B1	11	8	Proliferati	Carcinoma	Moderate	2	2
2		1	6 mo	1	6 mor	2	2	2	9.00pm	10.30	G2R2B1	7	4	Broad bas	Left vocal	Benign vo	2	2
2		2		2		2	2	2	9.00pm	10.00	G2R1B0	15	8	broad bas	right voca	benign vo	2	2
2		2		2		2	2	2	9.00pm	9.30 p	G2R1B0	5	2	Exophytic	carcinoma	moderate	2	2
2		2		2		2	2	2	8.00 pn	9.00p	G3R2B1	9	12	Irregular s	transglott	Poorly dif	2	2
2		2		1	5 yea	2	2	2	10.00pr	10.30	G3R1B2	12	8	Proliferati	transglott	well diffe	2	2
1	3 yea	2		2		2	2	2	9.00 pn	10.00	G2R2B1	10	4	Haemorrh	right voca	Benign vo	2	2
2		2		2		2	2	2	9.00 pn	11.00	G3R2B3	9	7	Extensive	Vocal cord	Keratosis	2	2
2		2		2		2	2	2	9.30 pn	10.30	G1R1B0	14	4	Exophytic	Carcinoma	moderate	2	2
2		2		2		2	2	2	11.00 p	12.00	G2R2B0	10	3	Bilateral h	Bilateral v	Benign ep	2	2
2		2		2		2	2	2	9.30pm	10.30	G3R3B1	10	8	Proliferati	Carcinoma	moderate	2	2
2		2		2		2	2	2	10.00pr	12.00	G3R2B0	14	7	Proliferati	Carcinoma	moderate	2	2
2		1	10 ye	2		2	2	2	10.00pr	10.30	G3R2B0	15	4	Irregular g	Carcinoma	well diffe	2	2
2		2		2		2	2	2	10.00pr	11.30	G2R2B0	9	6	Broad bas	vocal cord	Polyp with	2	2
2		2		1	5 yea	2	2	2	9.00pm	10.30	G3R2B2	8	5	Irregular l	Laryngeal	Necrotisir	2	2
2		2		1	2 yea	2	2	2	10.00pr	10.30	G2R1B0	10	9	Haemorrh	Right voca	Benign po	2	2
2		2		2		2	2	2	9.30pm	10.30	G3R2B1	12	2	Proliferati	transglott	well to mo	2	2
1	20 ye	2		2		2	2	2	9.30 pn	11.00	G2R1B0	15	8	Pedunculá	Right voca	Benign po	2	2
2		2		2		2	2	2	9.00 pn	10.00	G2R1B0	8	1	Broad bas	right voca	Benign vo	2	2
2		1	3 yea	2		2	2	2	10.00pr	11.00	G1R1B0	10	8	Ulceropro	Carcinoma	moderate	2	2
2		2		2		2	2	2	9.30 pn	11.00	G2R1B1	14	9	Small broa	Right voca	Benign vo	2	2
2		2		2		2	2	2	9.00pm	11.00	G3R2B1	8	8	Large ped	Left vocal	Benign vo	2	2
1	60-10	1	2 yea	2		2	2	2	10.00pr	11.00	G3R3B2	11	5	Ulceropro	transglott	moderate	2	2
2		2		2		2	2	2	10.00pr	12.00	G2R1B0	10	3	Ulceropro	Carcinoma	moderate	2	2
2		2		2		2	2	2	10.00pr	10.30	G2R2B1	15	7	Large ped	left vocal	benign vo	2	2
1	60 m	2		2		2	2	2	10.00pr	10.30	G2R1B1	17	8	Sessile br	right voca	benign vo	2	2
2		2		2		2	2	2	10.00pr	10.30	G2R2B2	14	8	Polyps at t	Bilateral v	Benign vo	2	2
2		2		1	3 yea	2	2	2	9.30 pn	10.30	G2R1B1	7	4	Reddish p	Right voca	Benign vo	2	2
2		2		2		2	2	2	9.30 pn	10.30	G3R3B1	11	3	polyp at th	right voca	Benign vo	2	2